"EFFECT OF NON SURGICAL PERIODONTAL THERAPY ON THE MICRONUCLEI FREQUENCY, SERUM MALONDIALDEHYDE AND SERUM HIGH SENSITIVE C – REACTIVE PROTEIN LEVELS IN CHRONIC PERIODONTITIS PATIENTS WITH AND WITHOUT WELL CONTROLLED TYPE 2 DIABETES MELLITUS"

DISSERTATION

Submitted to The Tamil Nadu Dr. M.G.R Medical University

in partial fulfillment of the requirement for the degree of

MASTER OF DENTAL SURGERY

BRANCH II

PERIODONTOLOGY

2016 – 2019

CERTIFICATE

This is to certify that the dissertation titled **"Effect of Non-Surgical Periodontal Therapy on the Micronuclei Frequency, Serum Malondialdehyde and Serum High Sensitive C – Reactive Protein Levels in Chronic Periodontitis patients with and without well controlled Type 2 Diabetes Mellitus"** is a bonafide record of the work done by **Dr. Blessing Emmanuel P**, Post graduate student during the period 2016-2019 under our guidance and supervision. The dissertation is submitted to **THE TAMIL NADU DR M.G.R MEDICAL UNIVERSITY, CHENNAI,** in partial fulfillment of the requirement for the award of the Degree of **MASTER OF DENTAL SURGERY IN PERIODONTOLOGY, BRANCH II.** It has not been submitted (partial or full) for the award of any other degree or diploma.

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DECLARATION

I hereby declare that this dissertation entitled **"Effect of Non-Surgical Periodontal Therapy on the Micronuclei Frequency, Serum Malondialdehyde and Serum High Sensitive C – Reactive Protein Levels in Chronic Periodontitis patients with and without well controlled Type 2 Diabetes Mellitus"** is a bonafide research work undertaken by me and that this thesis or a part of it has not been presented earlier for the award of degree, diploma, fellowship, or similar title of recognition.

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‘Not by might nor by power, but by my Spirit,’ says the Lord Almighty.

- The Bible

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CONTENTS

LIST OF ABBREVIATIONS

ABL	-	Alveolar bone loss
AGES	-	Advanced glycation end products
AgP	-	Aggressive periodontitis
ANOVA	-	Analysis of variance
BMI	-	Body mass index
BI	-	Bleeding index
CAL	-	Clinical attachment level
CAD	-	Coronary artery disease
CBMN	-	Cytokinesis block micronuclei
CP	-	Chronic periodontitis
CRP	-	C-Reactive protein
CVD	-	Cardiovascular diseases
CVS	-	Cardiovascular system
DMCP	-	Diabetes mellitus chronic periodontitis
DM	-	Diabetes mellitus
DNA	-	Deoxyribo nuclei acid
ELISA	-	Enzyme linked immuno sorbent assay
GAP	-	Generalized aggressive periodontitis
GCF	-	Gingival crevicular fluid
GI	-	Gingival index
HbA1c	-	Glycated haemoglobin
HCL	-	Hydrochloric acid
HDL	-	High density lipoprotein
Hs-CRP	-	High sensitive C-reactive protein

H_2O_2	-	Hydrogen peroxide
KCL	-	Potassium chloride
LDL	-	Low density lipoprotein
LPO	-	Lipid peroxidation
IL-6	-	Interleukin - 6
MDA	-	Malondialdehyde
MN	-	Micronuclei
MNF	-	Micronuclei frequency
NA	-	Nuclear abnormalities
NO	-	Nitric oxide
PD	-	Periodontal disease
PHA	-	Phytohaemagglutinin
PPD	-	Probing pocket depth
qPCR	-	Quantitative polymerase chain reaction
RBC	-	Red blood cell
RBS	-	Random blood sugar
ROS	-	Reactive oxygen species
SOD	-	Superoxide dismutase
SRP	-	Scaling and root planing
TAOC	-	Total antioxidant capacity
TBA	-	Thiobarbituric acid
TCA	-	Trichloroacetic acid
TNF-α	-	Tumour necrosis factor - α
TOS	-	Total oxidant status
UNC	-	University of North Carolina
VLDL	-	Very low density lipoprotein

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Abstract

BACKROUND

The over-production of reactive oxygen species (ROS) associated with the pathogenesis of several disorders such as diabetes mellitus (DM) and periodontal disease (PD) can contribute to injury of the host tissue, significant impairment to cell integrity and can cause oxidative damage to a large number of molecules such as carbohydrates, lipids and DNA. Because the oxidative stress is intrinsically related to the pathogenesis of type 2 diabetes, dyslipidemia and PD, and it lead to DNA damage. One of the most established methods for evaluating DNA damage is the micronucleus (MN) test. The ROS-mediated tissue destruction could be measured by the final product of lipid peroxidation (LPO), such as malondialdehyde (MDA). C-reactive protein (CRP) an acute-phase protein found in the blood, the levels of which rise in response to inflammation is linked closely to diabetes and periodontal infection.

AIM OF THE STUDY

The aim of the study was to evaluate the effect of non-surgical periodontal therapy on the micronuclei frequency, serum Malondialdehyde and serum Hs – CRP levels in chronic periodontitis patients with and without well controlled type 2 diabetes mellitus and healthy controls.

OBJECTIVES

(i) To assess the micronucleus frequency (MNF), as a biomarker for DNA damage in peripheral blood, in chronic periodontitis patients, with and without well controlled type 2 diabetes mellitus and healthy controls.

(ii) To evaluate and compare free radical damage in the form of serum malondialdehyde (MDA) levels in chronic periodontitis patients, with and without well controlled type 2 diabetes mellitus and healthy controls.

(iii) To assess the serum high sensitive C – Reactive protein level in chronic periodontitis patients, with and without well controlled type 2 diabetes mellitus and healthy controls.

MATERIALS AND METHODS

This was an interventional study including a total of 83 participants (28 chronic periodontitis with well controlled type DM, 27 chronic periodontitis and 28 healthy controls) of which 75 patients completed the three months foloow-up protocol. Clinical parameters (PI, GI PPD and CAL) and laboratory parameters (RBS, total cholesterol, triglycerides, CBMN frequency, HbA1c (%), MDA, and Hs-CRP) were recorded at baseline and three months following non-surgical periodontal therapy.

RESULTS

In the present study at baseline all the periodontal parameters like PI, GI, PPD, and CAL were elevated in group 1 and group 2 than group 3. There was no statistical difference in PI, GI at baseline among three groups. In terms of mean PPD and mean CAL was more in DM group compared to CP group. In other words chronic periodontitis with well controlled type 2 DM had more periodontal breakdown than in patients with chronic periodontitis alone. This shows that DM has additional effect on periodontium. 3 months after SRP all the clinical parameters are reduced significantly in all the groups. Similarly laboratory parameters like RBS, total cholesterol, triglycerides, CBMN frequency, MDA, and Hs-CRP levels were

elevated in group 1 and group 2 than group 3. These values significantly declined three months after non-surgical periodontal therapy.

CONCLUSION

Mean CBMN frequency, MDA, and Hs-CRP levels were elevated in individuals with chronic periodontitis and chronic periodontitis patients with well controlled type 2 diabetes mellitus. This study provides evidence that non-surgical periodontal therapy contributes to reduction in CBMN frequency, serum MDA, and serum Hs-CRP levels in these patients. Larger clinical trials are needed to confirm these findings.

Introduction

Periodontitis is a term used to describe an inflammatory process, initiated by plaque biofilm, that leads to loss of periodontal attachment to the root surface and adjacent alveolar bone and which ultimately results in tooth loss. The gram-negative anaerobic or facultative bacteria within the subgingival biofilm are the primary etiologic agents but the majority of periodontal destruction occurs due to an inappropriate host response to this pathogens.[1]

A diverse range of endogenous chemical mediators organize the host response and controls the inflammatory response. These chemical signals regulate the traffic of leukocytes and control the leukocyte response. The classic eicosanoids such as prostaglandins and leukotrienes exert a wide range of actions and play a key role in inflammation. The chemical mediators include lipid derived mediators, cytokines, chemokines, reactive oxygen species (ROS), reactive nitrogen species etc.[2]

The initial host response is the release of reactive oxygen species (ROS) via the metabolic process of respiratory burst in the Polymorphonuclear leukocytes (PMNL's), macrophages and monocytes. Excessive production of these ROS results in oxidative stress in the body. Reactive oxygen species (ROS) have a very short life (10^{-6}-10^{-9} seconds), so it is not easy to detect its presence. The ROS-mediated tissue destruction could be measured by the final product of lipid peroxidation (LPO), such as Malondialdehyde (MDA).[1]

No longer can periodontal diseases be considered simple bacterial infections. Rather, they are complex diseases of multifactorial nature involving an intricate interplay between the subgingival microbiota, the host immune and inflammatory

responses, and environmental modifying factors.[3] Thus, periodontal health must not be considered solely in the context of plaque/bacteria levels and control but must embrace a holistic consideration and evaluation of all factors responsible for the emergence of disease, as well as the restoration and maintenance of health.[4]

The relevance of recognizing such important determinants of periodontal health and disease as controllable and uncontrollable predisposing and modifying factors cannot be underestimated, and their assessment for each patient is crucial to attaining and maintaining clinical periodontal health. Diabetes mellitus has, for many years, been recognized as an important risk factor for periodontal diseases and associated with significantly higher prevalence and severity of periodontitis.[5]

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia and insufficiency of secretion or action of endogenous insulin and is an established risk factor for periodontitis[6]. There are two main types of diabetes (i) Type 1 diabetes mellitus and (ii) Type 2 diabetes mellitus. More recent data have confirmed a significant association between chronic hyperglycemia and a high prevalence of severe periodontitis[6, 7]. Furthermore, in many diabetic patients DM is undiagnosed, and the prevalence of these individuals is increasing. Hence, DM represents an enormous public health challenge and is by far the principal systemic disease affecting periodontitis in terms of extent of population affected.[8] In addition, there is accumulating evidence that periodontal inflammation may itself contribute to the onset and persistence of hyperglycemia, in that inflammation is associated with poorer glycemic control in individuals with DM and may be associated with an increase in incident DM in longitudinal prospective studies.[9]

A factor associated with complications of DM is the process of non-enzymatic glycation of proteins, lipids and nucleic acids, with subsequent formation of advanced glycation end products (AGEs). One of the main consequences of this adverse action is the formation of oxidative stress.[10] ‘‘Oxidative stress’’ can be defined as any disturbance in the balance of antioxidants and pro-oxidants in favor of the later due to different factors such as aging, drug actions and toxicity, inflammation and/or addiction.[11] Oxidative stress induces cellular damage and insulin resistance, and emerges as the major mechanisms for related co-morbidities. In addition, patients with type 2 diabetes present a deficient antioxidant defense compared to normal individuals. The increased production of the ROS has also been assigned to the glycation of proteins and/or auto-oxidation of glucose during the process of hyperglycaemia. Therefore, besides to the AGEs, the oxidative stress presents an important role in the pathogenesis and complications of DM.[10]

DM is well recognized as a risk factor for periodontal disease. Diabetic individuals present an increased prevalence, severity and progression of periodontitis, when compared to non-diabetic individuals. Subjects with type 2 DM are 2.8 times more likely to have destructive periodontal disease and 4.2 times more likely to have significant alveolar bone loss compared to systemically healthy subjects. Periodontal disease was proposed to be the sixth complication of DM.

The over-production of ROS associated with the pathogenesis of several disorders such as DM, dyslipidemia and periodontal disease, can contribute to injury of the host tissue, significant impairment to cell integrity and can cause oxidative damage to a large number of molecules such as carbohydrates, lipids and DNA. Because the oxidative stress is intrinsically related to the pathogenesis of type 2

diabetes, dyslipidemia and PD, and it lead to DNA damage. One of the most established methods for evaluating DNA damage is the micronucleus (MN) test.[12]

Micronuclei (MN) and other nuclear anomalies such as nucleoplasmic bridges and nuclear buds are biomarkers of genotoxic events and chromosomal instability. These genome damage events can be measured simultaneously in the cytokinesis block micronucleus cytome (CBMN) assay.

The MN test analyses is based on the identification of a secondary nucleus (MN), which is originated from acentric chromosome fragments, acentric chromatid fragments, or whole chromosomes that fail to be included in the daughter nuclei at the completion of telophase during mitosis because they did not attach properly with the spindle during the segregation process in anaphase. These displaced chromosomes or chromosome fragments are eventually enclosed by a nuclear membrane and except for their smaller size are morphologically similar to nuclei after conventional nuclear staining. Previous studies suggested that periodontal treatment may be effective in maintaining systemic health by decreasing circulating ROS.[12]

The hyperglycaemia associated to uncontrolled DM increases the formation of AGEs, including low density lipoprotein (LDL)-AGE, and is associated with the largest amount of LDL-oxidised. Both AGEs and LDL-oxidised, present at the same time in the blood of patients with DM, have several biological effects on lymphocytes and monocytes. These cells are involved in the pathogenesis of various diseases associated with DM, such as lower resistance to infections, vascular disease and greater severity of the periodontitis.[10]

C-reactive protein (CRP) an acute-phase protein found in the blood, the levels of which rise in response to inflammation is linked closely to diabetes and periodontal infection. All studies on periodontitis patients with diabetes clearly demonstrate that the inflammatory response in such patients is related to increase in CRP.[13] Recent investigations emphasized the role of moderate elevation of CRP levels as a risk factor for cardiovascular diseases.[14] Studies suggested that non-surgical periodontal therapy significantly suppress the serum C-reactive protein level in chronic periodontitis patients with type 2 diabetes mellitus.[15]

Elevated concentrations of a variety of oxidative stress markers were linked with a more frequent occurrence of cardiac events. High levels of micronuclei have been detected to be significantly correlated with Type 2 diabetes mellitus as well as with the occurrence and the severity of coronary artery disease (CAD). An elevated level of MDA indicates increase in production of oxygen free radicals suggesting the possible risk in atherogenesis, leading to coronary heart diseases[16]. There is strong evidence that inflammation plays a key role in the cascade of atherosclerosis, from its beginning to development of instability and plaque rupture. Raised levels of inflammatory mediators particularly CRP predict cardiovascular events. HsCRP is a powerful marker of vascular risk in a wide range of populations and, as such, is a powerful research tool in assessing at-risk populations. Determination of these markers contributes to understanding the development of and prevention of CVS disorders.[17] All three bio markers include Micronuclei, Malondialdehyde, and HsCRP will be higher in patients with periodontitis, Type 2 diabetes mellius and cardiovascular diseases. Non-surgical periodontal therapy includes scaling and root planing will reduce the bacterial load thus reduce the amount of inflammation.

Various studies suggested that non-surgical periodontal therapy effectively reduce the levels of MDA and hs-CRP thus reducing the cardiovascular risk. **Kanduluru et al 2014**[18] showed that nonsurgical periodontal treatment resulted in lower glycemic levels and the reduction of clinical parameters of periodontal infection, confirming the existing relation between type 2 DM and periodontal disease. **Katagiri et al 2009**[15] demonstrated that periodontal treatment with topical antibiotics was effective towards the improvement of glycemic control in type 2 diabetic patients with periodontal disease when associated with a decrease in serum hs-CRP.

The present study designed to compare the micronuclei frequency in peripheral blood, serum Malondialdehyde level, and serum high sensitive C - reactive protein at baseline and 3 months after non-surgical periodontal therapy in chronic periodontitis patients with and without well controlled type 2 diabetes mellitus, and in patients with healthy periodontium.

Aims & Objectives

Aim of the study

The aim of the study was to evaluate the effect of non-surgical periodontal therapy on the micronuclei frequency, serum Malondialdehyde and serum Hs – CRP levels in chronic periodontitis patients with and without well controlled type 2 diabetes mellitus and healthy controls.

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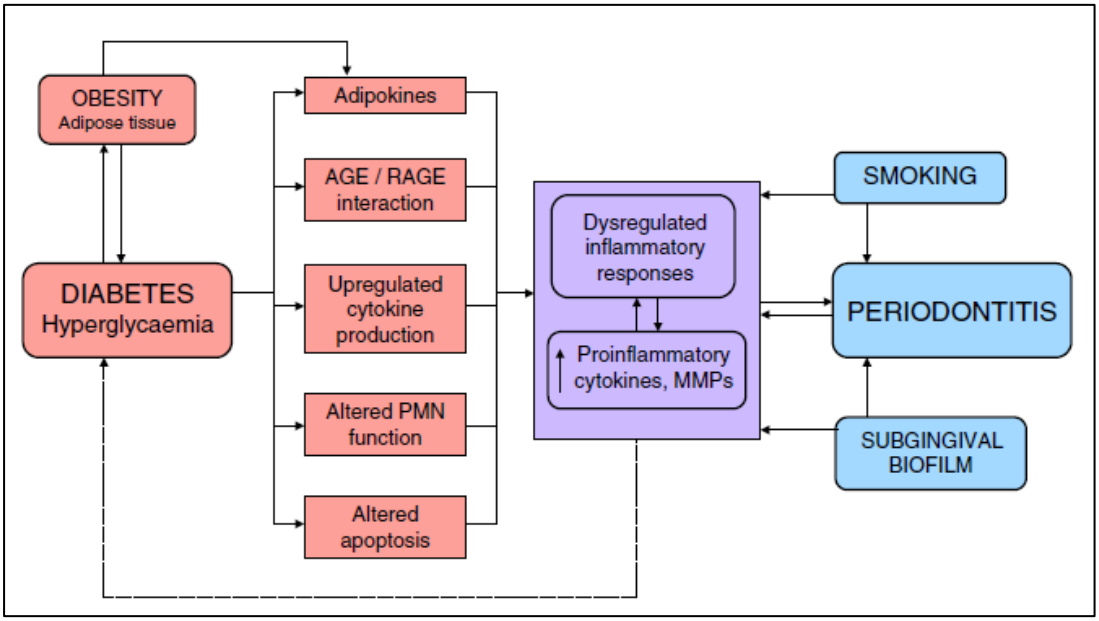
Review of Literature

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia and insufficiency of secretion or action of endogenous insulin and is an established risk factor for periodontitis.[6] There are two main types of diabetes (i) Type 1 diabetes mellitus and (ii) Type 2 diabetes mellitus. More recent data have confirmed a significant association between chronic hyperglycemia and a high prevalence of severe periodontitis.[6,7] Furthermore, in many diabetic patients DM is undiagnosed, and the prevalence of these individuals is increasing. Hence, DM represents an enormous public health challenge and is by far the principal systemic disease affecting periodontitis in terms of extent of population affected.[8] In addition, there is accumulating evidence that periodontal inflammation may itself contribute to the onset and persistence of hyperglycemia, in that inflammation is associated with poorer glycemic control in individuals with DM and may be associated with an increase in incident DM in longitudinal prospective studies.[9]



Exacerbated and dysregulated inflammatory responses are at the heart of the proposed two-way interaction between diabetes and periodontitis, and the hyperglycaemic state results in various proinflammatory effects that impact on multiple body systems, including the periodontal tissues. Adipokines produced by adipose tissue include proinflammatory mediators such as TNF-α, IL-6 and leptin. The hyperglycaemic state results in deposition of AGEs in the periodontal tissues (as well as elsewhere in the body), and binding of the receptor for AGE (RAGE) results in local cytokine release and altered inflammatory responses. Neutrophil function is also altered in the diabetic state, resulting in enhancement of the respiratory burst and delayed apoptosis (leading to increased periodontal tissue destruction). Local production of cytokines in the periodontal tissues may, in turn, affect glycaemic control through systemic exposure and an impact on insulin signalling. All of these factors combine to contribute to dysregulated inflammatory responses that develop in the periodontal tissues in response to the chronic challenge by bacteria in the subgingival biofilm, and which are further exacerbated by smoking.[19]

The hyperglycaemia associated to uncontrolled DM increases the formation of AGEs, including low density lipoprotein (LDL)-AGE, and is associated with the largest amount of LDL-oxidised. Both AGEs and LDL-oxidised, present at the same time in the blood of patients with DM, have several biological effects on lymphocytes and monocytes. These cells are involved in the pathogenesis of various diseases associated with DM, such as lower resistance to infections, vascular disease and greater severity of the periodontitis.[10]

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MICRONUCLEI IN PERIODONTITIS AND DIABETES MELLITUS

Periodontal tissue destruction and local inflammation were significantly more severe in diabetics. Frequency of binucleated cells with MN and MNF, as well as nucleoplasmic bridges, were significantly higher for poor controlled diabetics with dyslipidemia and PD in comparison with those systemically healthy, even after adjusting for age, and considering Bonferroni's correction. Elevated frequency of micronuclei was found in patients affected by type 2 diabetes, dyslipidemia and PD. This result suggests that these three pathologies occurring simultaneously promote an additional role to produce DNA impairment. In addition, the micronuclei assay was useful as a biomarker for DNA damage in individuals with chronic degenerative diseases.

There is an increasing effort worldwide to determine the impact of environmental, genetic and life-style factors on genomic stability in human populations. One technique that has been adopted by numerous laboratories is the measurement of micronuclei (MN) in peripheral blood lymphocytes, epithelial cells, erythrocytes and fibroblasts.[20]

Srinivasan et al 2017 demonstrate cytologic changes of the exposed oral epitheial cells using H&E staining, concluded that micronuclei frequency, enlarged nucleus, perinuclear halo, binucleation, cytoplasmic streaks, candida and inflammation were found to be statistically significant.[21]

Rathod et al 2016 assess the micronucleus frequency (MNF), as a biomarker for DNA damage, in individuals with type 2 Diabetes Mellitus and chronic periodontitis. A total of 60 subjects were recruited for the study and divided into three groups. 20 subjects of group 1 had diabetes mellitus along with chronic periodontitis, Subjects with Diabetes mellitus and healthy periodontium were allotted to group 2 and group 3 include Subjects without Diabetes melilitus and with Chronic Periodontitis. Periodontal clinical examination was done. Blood sample collected was use to prepare a slide which was fixed in 5% giemsa solution and was analyzed in microscope then scoring of micronuclei was done. The mean micronuclei observed in group 1, 2 and 3 were 14.8, 11 and 10.85 respectively i.e. group 1 shows significantly greater damage than other two groups. It was concluded that CBMN method was useful as a biomarker for DNA damage in individuals with chronic degenerative systemic diseases such as type 2 DM, as well as chronic local disease, such as PD.[12]

Zamora Perez et al 2015 evaluate DNA and oxidative damage in subjects with chronic or aggressive periodontitis and healthy controls. Buccal mucosa cells and whole saliva were collected from 160 subjects, who were divided into three groups: subjects with chronic periodontitis (CP) (n = 58), subjects with aggressive periodontitis (AgP) (n = 42) and a control group (n = 60). DNA damage was determined by counting micronuclei (MN) and nuclear abnormalities (NAs) in exfoliated cells, including binucleated cells, cells with nuclear buds and karyolitic, karyorrhectic, condensed chromatin and pyknotic cells. The degree of oxidative stress was determined by quantifying 8-hydroxy-20-deoxyguanosine (8-OHdG) in whole saliva. Subjects with CP or AgP presented significantly more ($p < 0.05$) MN and NAs and higher levels of 8-OHdG ($p < 0.05$) compared with the control group. Their results indicate that subjects with periodontitis (CP or AgP) exhibited an increase in the frequency of MN, NAs and 8-OHdG, which is directly related to DNA damage. In addition, a positive correlation exists between oxidative stress produced by periodontitis disease and MN.[22]

Corbi et al 2014 assess the micronucleus frequency (MNF), as a biomarker for DNA damage, in individuals with type 2 DM, dyslipidemia and PD. One hundred and fifty patients were divided into five groups based upon diabetic, dyslipidemic and periodontal status (Group 1 – poor controlled DM with dyslipidemia and PD; Group 2 – well-controlled DM with dyslipidemia and PD; Group 3 – without DM with dyslipidemia and PD; Group 4 – without DM, without dyslipidemia and with PD; and Group 5 – without DM, dyslipidemia and PD). Blood analyses were carried out for fasting plasma glucose, HbA1c and lipid profile. Periodontal examinations were performed, and venous blood was collected and

processed for micronucleus (MN) assay. The frequency of micronuclei was evaluated by cell culture cytokinesis-block MN assay.[10]

Bastos-Aires et al 2013 characterize the frequency of micronuclei according to the periodontal status. For this purpose, we analysed oral exfoliated cells of 30 patients matched by age and sex (15 control patients with healthy periodontum to mild periodontitis and 15 patients with moderate to severe periodontitis). Our results indicated a 2.3-fold increase in MN basal levels in patients with moderate to severe periodontitis compared to the control patients ($P < 0.001$), suggesting that the periodontal status may affect MN reference levels. Periodontal disease can cause changes in baseline levels of MNs. These preliminary results indicate that a better characterization of oral health factors, which might influence MN levels in oral epithelial cells.[23]

Avula et al 2012 in their study Micronuclei frequency was analyzed in the peripheral lymphocytes of 10 patients with chronic periodontitis (CP), 10 patients with generalized aggressive periodontitis (GAP) and 10 healthy controls. Various clinical parameters like the probing depth, clinical attachment level, and percentages of sites with bleeding and plaque were recorded. The results indicated that the cytogenetic damages in the periodontitis groups were not different from those in the control group.[24]

Shettigar et al 2012 evaluated the induction of micronuclei due to increased glycosylation in type 2 diabetes. Forty-nine subjects divided into two groups of normoglycemic controls and type 2diabetic cases were recruited in the study. Whole blood was cultured and micronuclei were scored in all the cases. This

was correlated with age, sex, blood glucose levels and glycosylated hemoglobin. Age and sex matched diabetic patients had an increased micronuclei frequency in response to elevated glycosylation of haemoglobin compared to normoglycemic subjects. The increased glycosylation seems to induce oxidative damage in the DNA of the diabetic patients, which manifests as an increased micronuclei frequency. This has a potential to be used as a biomarker for subsequent diabetic complications.[25]

Zuniga-Gonzalez et al 2007 demonstrated that either controlled (glycosylated haemoglobin levels 7%) or uncontrolled diabetic patients (glycosylated haemoglobin levels.7%) had 2-fold higher frequency of MNs in buccal mucosa samples than healthy subjects.[26]

Martinez Perez et al 2007 A case–control study was carried out on a sample of 15 Mexican patients (40–56 years old) with type 2 diabetes mellitus (DM2) that had developed five years and been treated with oral hypoglycemic drugs (sulfonylurea and/or metformin), with no microvascular or macrovascular complications. Result showed a MN frequency significant increase in DM2 patients (6.53 ± 2.03 per 1000 cells) relative to that of the control group (3.10 ± 1.79 per 1000 cells). MN may constitute a possible component of a panel of biomarkers for the risk of DM2.[27]

MDA IN PERIODONTITIS AND DM

Periodontitis is a term used to describe an inflammatory process, initiated by plaque biofilm, that leads to loss of periodontal attachment to the root surface and adjacent alveolar bone and which ultimately results in tooth loss. The gram-negative anaerobic or facultative bacteria within the subgingival biofilm are the primary

etiologic agents but the majority of periodontal destruction occurs due to an inappropriate host response to these pathogens. Polymorphonuclear leukocytes (PMNs) are the primary mediators of host response against pathogens and they generate increased levels of reactive oxygen species (ROS). Reactive oxygen species (ROS) is a term collectively describing oxygen free radicals and other non-radical oxygen derivatives involved in oxygen radical production. Reactive oxygen species (ROS) have a very short life (10^{-6}-10^{-9} seconds), so it is not easy to detect its presence. The ROS-mediated tissue destruction could be measured by the final product of lipid peroxidation (LPO), such as malondialdehyde (MDA). Oxidative stress is implicated in various pathological conditions such as rheumatoid arthritis, DM, and Periodontitis.[1]

Trivedi et al 2014 evaluate and compare free radical damage in the form of malondialdehyde (MDA) levels in whole saliva and plasma in diabetic chronic periodontitis patients and systemically healthy chronic periodontitis patients. Sixty CP patients (30 type 2 diabetics [DMCP] and 30 systemically healthy [CP]) and 60 periodontally healthy (30 type 2 diabetics [DMPH] and 30 systemically healthy [PH]) were included in the study. After clinical measurements, blood and saliva samples were collected. SOD, GR, and CAT activities in red blood cell (RBC) lysate and saliva and MDA levels in plasma and saliva samples were spectrophotometrically assayed. ANOVA test followed by post-hoc test was used to compare the intra and inter group variances amongst the study groups. MDA levels in both the periodontitis groups were higher than periodontally healthy groups but the difference between CP and DMCP groups did not reach statistical significance ($p>0.05$). This study favors the role of oxidative stress in both diabetes and

periodontitis. It shows that the compensatory mechanism of the body is partially collapsed due to excessive production of free radicals during periodontitis, and is not able to cope with increased free radical generation due to diabetes thereby worsening the situation.[1]

A meta-analysis by **Liu et al 2014** show that periodontitis patients had higher MDA levels than periodontal healthy controls (SMD = 0.99; 95% CI: 0.12, 1.86; *P* = 0.026). This meta-analysis systematically summarized the results of 16 independent studies from different countries and suggested that oxidative stress biomarkers TAOC levels in peripheral blood were lower and MDA and NO levels in peripheral blood were higher in periodontitis patients than healthy subjects, which indicated an elevation in systemic oxidative stress status in periodontitis patients. This evidence suggested that chronic periodontitis was associated with systemic oxidative stress in human bodies. Our findings further indicated that clinical intervention of periodontitis may be beneficial for periodontitis patients systemic oxidative stress control and reduce its potential effect to systemic diseases.[28]

Al-Rawi et al 2011 assess the salivary content of lipid peroxidation and antioxidants in patients with type 2 diabetes. They studied 25 patients with type 2 diabetes and other 25 age- and sex-matched health control. To evaluate the oxidative status we measured the levels of malondialdehyde (MDA) in the saliva and serum of all participants. Lipid profile was also estimated through measuring total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and very low-density lipoprotein cholesterol (VLDL-C) levels. Antioxidant levels were also assessed through measuring the salivary and serum concentration of uric acid, superoxide dismutase and reduced glutathione (GSH).

The high concentration of lipid fractions in saliva usually follows that recorded in serum. Salivary MDA levels, a product of lipid peroxidation, were significantly increased among diabetics together with uric acid. However, GSH levels were similar to those of the control group. In this study concluded that Lipid peroxidation and antioxidant parameters assessed in saliva of diabetic patients may be of great importance in evaluating the disease activity and severity. The increase in lipid peroxidation and the tendency of antioxidants to rise in diabetes is probably due to an adaptive response to the pro-oxidant status of diabetes.[29]

Akalin et al 2007 investigated MDA levels and total oxidant status (TOS) in serum, saliva and gingival crevicular fluid (GCF) in patients with chronic periodontitis (CP). Thirty-six CP patients and 28 periodontally healthy controls were included in the study. Following clinical measurements and samplings, MDA and TOS levels were measured by high-performance liquid chromatography and a novel automatic colorimetric method, respectively. While the saliva and GCF MDA levels and serum, saliva and GCF TOS values were significantly higher in the CP group than the control group, no significant difference in serum MDA levels was found. Strong positive correlations were observed between periodontal parameters and MDA and TOS levels. The results revealed that LPO significantly increased locally in the periodontal pocket/oral environment, while TOS displayed both systemic and local increases in periodontitis. The findings suggest that increased LPO and TOS may play an important role in the pathology of periodontitis, and are closely related to the clinical periodontal status.[30]

Hs-CRP IN PERIODONTITIS AND DM

The role of periodontal disease in the etiology of cardiovascular disease (CVD) has recently received considerable attention. Several observational epidemiologic studies have found that poor periodontal health status is associated with an increased risk for CVD.[31]

C-reactive protein an acute-phase protein found in the blood, the levels of which rise in response to inflammation is linked closely to diabetes and periodontal infection. In various diseases that result in tissue injury or inflammation, the CRP levels may rise within hours after an acute event. It is though that elevated CRP levels assist in complement binding to foreign and damaged cells and enhance the humoral response to the disease.

The function of immune cells including neutrophils is decreased in diabetes but the monocyte/macrophage cell line may be hyper responding when faced with bacterial antigenic contact. This hyper response results in a greater production of pro-inflammatory cytokines as tumor necrosis factor alpha and IL-6, which result in elevation of CRP levels. The measurement of elevated CRP levels in periodontitis and diabetes are helpful in clinical diagnosis, therapy and monitoring of inflammatory conditions.[13]

Gupta et al 2017 assessed the levels of CRP in patients with and without periodontitis and their relation with BMI and smoking behaviour. Total 31 subjects in each group: Case (periodontitis) and Control (without periodontitis) were selected using convenience sampling technique. All subjects underwent periodontal examination by a single examiner. Serum CRP samples were taken before

periodontal treatment. Results showed increase in mean CRP levels in periodontitis (5.8595mg/L) with high statistical significance (P=0.000) in comparison to subjects without periodontitis (1.1214mg/L) was observed. BMI showed positive association with periodontitis (P=0.046) but not with CRP (0.213). Smoking behaviour showed no significant relation with either CRP (P=0.344) or periodontitis (P=0.541). They found highly significant association between periodontitis and CRP levels but not always with BMI and smoking. CRP, which is an established marker for CVD was significantly increased in periodontal infections. Hence, a close interaction among Physician, Periodontist and Patient to prevent adverse health situations is recommended.[32]

Martu et al 2017 investigated the serum C-reactive protein (CRP) values in the presence of A.actinomycetemcomitans, P. gingivalis, T. denticola or T. forsythia bacteria, as an indicator of the cardiovascular risk. The study consisted of 64 male and female subjects, aged 55 to 75 years. Periodontal parameters were examined, serum CRP was analyzed, and Porphyromonas gingivalis, A. actinomycetemcomitans, T. forsythia and T. denticola from the subgingival bacterial plaque were detected by real-time quantitative polymerase chain reaction (qPCR). Pathogen prevalence rates were: 45.0% P. gingivalis; 20.5% A. actinomycetemcomitans; 86.1% T. forsythia; 86.3% T. denticola. The mean CRP was 1.5 mg / L. There was a significant difference in CRP values between subjects who had P. gingivalis compared to those without. There were no significant differences for any of the other pathogens. The presence of P. gingivalis was associated with a 1.20-fold increase in CRP. Of the four periodontal pathogens

investigated, only the presence of P. gingivalis in subgingival plaque samples was significantly associated with a high level of C-reactive protein.[33]

Anwar et al 2016 examined the plasma C-reactive protein levels in Pakistani population in association with periodontitis and diabetes. Patients were divided in four groups; group I (normal healthy), group 2 (periodontitis), group 3 (diabetes) and group 4 (both periodontitis and diabetes). Each group comprised 20 patients. Patients who have at least 15 teeth and age between 30-60 years were included. The obtained values of the patient samples and control sera were multiplied by dilution factor of 100 to obtain CRP results in mg/l. Statistically significant difference of C-reactive protein levels is present between periodontitis, diabetes and periodontitis diabetes group as compared to control group ($P<0.05$). They concluded that the CRP levels are raised with periodontitis and diabetes, and if both are present in a person, these levels are further elevated.[13]

Shojaee et al 2013 compared of the amount of salivary C - reactive protein (CRP) in healthy subjects and patients with periodontal disease. In this study subjects were divided into three groups of healthy (n = 30), gingivitis (n = 30), and chronic periodontitis (n = 30), based on Gingival Index (GI) and Clinical Attachment Loss (CAL) indices. 2ml saliva samples were collected from these people and clinical indicators including GI, CAL, Periodontal Pocket Depth (PPD), and Bleeding Index (BI) were assessed. ELISA method was used to evaluate the salivary CRP levels. The statistical analysis showed a significant difference in salivary CRP concentrations between the periodontitis patients and healthy subjects. The results indicate that there is a significant association between periodontitis and salivary CRP concentrations.[34]

Nakajima et al 2010 found out that the concentrations of hs-CRP and IL-6 in the sera of periodontitis patients was significantly higher than those in control subjects. They concluded that Although periodontal infection does affect the concentration of hs-CRP and IL-6 in serum, a subgroup of patients exist who are highly susceptible to an increased risk of CHD associated with periodontitis, suggesting that there may be subjects who have an elevated risk of CHD independent of susceptibility to periodontal tissue destruction per se.[35]

A systematic review and meta-analysis by **Paraskevas et al 2008** explored the robustness of observations that CRP is elevated in periodontitis. Similarly, the effect of periodontal therapy on CRP levels was investigated. In conclusion, this systematic review provides evidence that periodontitis elicits a mild acute-phase response with elevation of CRP levels compared with healthy controls. Periodontal treatment results in lowered CRP levels.[36]

Salzberg et al 2006 were working on elevated levels of C-reaction protein in periodontitis. The elevated inflammatory cytokines levels associated with destructive periodontal diseases cause an increase in CRP levels.[37]

Persson et al 2005 assess periodontal status and hsC-rp serum levels in consecutive subjects hospitalized and diagnosed with acute myocardial infarction (AMI) and in a group of carefully matched subjects (gender, age social, ethnic, and smoking habits) without clinical evidence of CVD. They found that the hs-CRP level to be above 10.0 mg/l in all the subjects in which evidence of significant alveolar bone loss was present indicating periodontitis. Thus, the level of CRP tends to increase with the periodontal destruction marked by ABL.[38]

Joshipura et al 2004 evaluated cross-sectionally the association between periodontal disease and C-reactive protein (CRP), fibrinogen, factor VII, tissue plasminogen activator (t-PA), LDL-C, von-Willebrand factor, and soluble tumor necrosis factor receptors 1 and 2. They concluded that periodontal disease showed significant associations with biomarkers of endothelial dysfunction and dyslipidemia, which may potentially mediate the association between periodontal and cardiovascular disease.[39]

Materials & Methods

STUDY DESIGN

This is a comparative interventional study for evaluating the Micronuclei frequency, serum Malondialdehyde levels and serum High sensitive C-reactive protein levels in chronic periodontitis patients with and without well controlled type 2 diabetes mellitus before and 3 months after scaling and root planning procedures.

STUDY SETTING

Chronic periodontitis patients with and without diabetes mellitus patients for the study were selected from the outpatient of department of periodontics, Sree mookambika institute of dental sciences, Kulasekaram.

STUDY DURATION

The study duration was of one year, which lasted from January 2018 to December 2018.

INFORMED CONSENT AND ETHICAL CONSIDERATIONS

The study protocol was approved by the institutional research committee of Sree mookambika institute of dental sciences (Ref No: 17/09/2017) and also by the Institutional Human Ethics Committee of Sree mookambika institute of medical sciences, Kulasekaram (SMIMS/IHEC No: 2/PROTOCOL No: 36/2017) and was registered under the Clinical trials Registry of India (CTRI/2018/09/015803).

Study protocol was explained to patient and the information on the nature and potential benefit of their participation in the study was also explained. Informed consent was obtained from all subjects after screening.

SAMPLE SIZE CALCULATION

Sample size was calculated based on the previous study by Rathod et al 2016.

The sample size $N=2\frac{\sigma^2}{\delta^2}(Z\alpha + Z\beta)^2$

$\alpha = 0.05$

$\beta = 0.20$

$Z\alpha = 1.96$

$Z\beta = 0.84$

$\sigma = 3.5$

$\delta = 3.0$

Power = $1-\beta = 80\%$

$N=2\frac{\sigma^2}{\delta^2}(Z\alpha + Z\beta)^2 = 15.7x\ \frac{12.25}{9} = 21.8$ rounded off to 25 for each group.

The number of participants required in each intervention group is 25.

Total sample size = 3x25 = 75

The selected patients were assigned into three groups, each group consisting of 25 patients

Group I – Twenty five chronic periodontitis patients with well controlled type 2 diabetes mellitus.

Group II – Twenty five chronic periodontitis without type 2 diabetes mellitus

Group III – Twenty five healthy control patients

INCLUSION CRITERIA

Group I – Twenty five chronic periodontitis patients with well controlled type 2 diabetes mellitus.

1. Patients with chronic periodontitis was diagnosed when there was two or more interproximal sites with CAL $\geq$ 4 mm, not on the same tooth, or two or more interproximal sites with PPD $\geq$ 5 mm, not on the same tooth. (Page and Eke 2007)

2. Patients with $\geq$ 20 teeth

3. Patients with clinical bleeding on probing

4. Patients were considered to be well controlled Type 2 diabetic when presenting with fasting blood glucose levels of 126mg/dL, and glycated hemoglobin (6.0 to 6.5%). [According to the criteria of American Diabetes Association 2010]

Group II – Twenty five chronic periodontitis without type 2 diabetes mellitus

1. Patients with chronic periodontitis was diagnosed when there was two or more interproximal sites with CAL $\geq$ 4 mm, not on the same tooth, or two or more interproximal sites with PPD $\geq$ 5 mm, not on the same tooth. (Page and Eke 2007)

2. Patients with $\geq$ 20 teeth

3. Patients with clinical bleeding on probing

Group III – Twenty five healthy control patients

1. Patients with clinical bleeding on probing
2. Patients with $\geq$ 20 teeth

EXCLUSION CRITERIA

Subjects will be excluded if

1) They suffer from any systemic condition (except Type 2 Diabetes mellitus) that could affect the progression of periodontal disease (immunological disorders), smokers and former smokers, alcoholics and former alcoholics, obese, pregnant and lactating women and those taking oral contraceptive drugs.

2) There is a presence of an active infection other than periodontitis.

3) There is an intake of antibiotics/ corticosteroids and or non-steroidal anti-inflammatory drugs during the previous 4 weeks.

4) Any previous history of benign or malignant tumours.

5) They had received professional periodontal treatment during the 6 month period prior to the study.

6) They had used mouth rinses containing antimicrobials in the preceding 2 months.

7) Subjects with periapical pathology, orthodontic appliances and multiple systemic complications of diabetes mellitus, inflammatory bowel disease, psoriasis, RA, Nephritis will also be excluded from the study.

CLINICAL PARAMETERS

Periodontal examination was conducted in the Department of Periodontics, Sree Mookambika institute of dental sciences, Kulasekaram. The following clinical parameters were assessed using UNC 15 probe **(Colour Plate – 1).** Clinical parameters were measured at six sites of all teeth (mesiobuccal, miduccal, distobuccal, mid lingual and distolingual) at baseline and 3 months following non-surgical periodontal therapy.

1. Plaque index(PI)
2. Gingival index (GI)
3. Probing pocket depth (PPD)
4. Clinical attachment level (CAL)

LABORATORY PARAMETERS

1. Micronuclei frequency (MN)

2. Serum Malondialdehyde (MDA) level
3. Serum High sensitive C-reactive protein level (Hs-CRP)
4. Glycated haemoglobin (HbA1C)
5. Random blood sugar (RBS)
6. Total cholesterol level
7. Triglycerides

ARMAMENTARIUM

The collection of blood and non-surgical periodontal therapy were performed with the following equipment's **(Colour Plate – 2)**

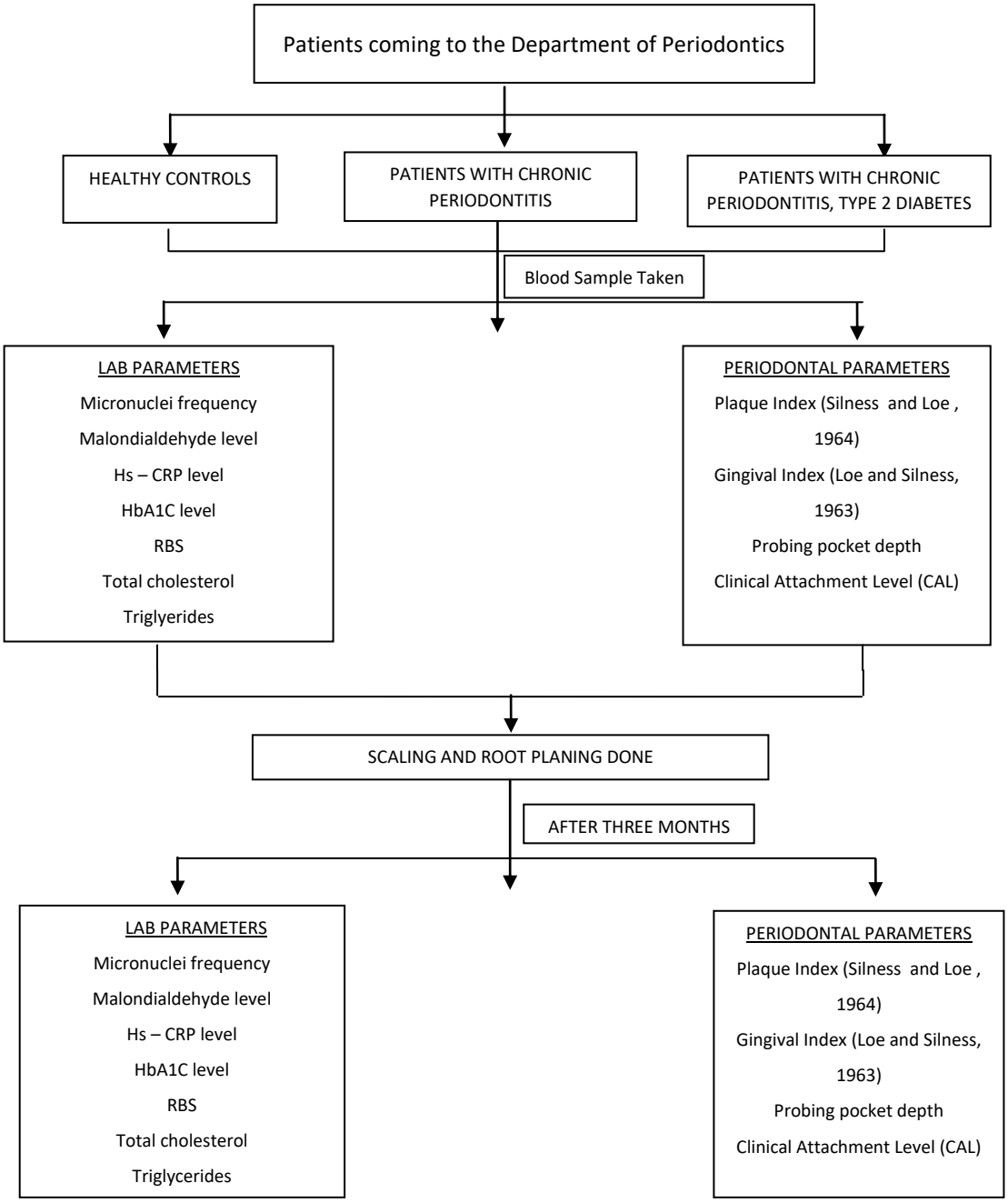
1. Mouth mirror
2. UNC 15 periodontal probe
3. Gracey area specific curette
4. 23 Gauge 6 ml disposable syringe
5. Clot activator tube
6. Vacutainer
7. REMI Laboratory centrifuge

PROCEDURE

After the patients were included in the study, the 6 ml of blood was collected by venepuncture **(Colour Plate – 3).** PI, GI, PPD, and CAL were recorded from each patient of the three groups. The clinical and laboratory parameters recorded at baseline were given as T0. After baseline evaluation all the patients received non-surgical periodontal therapy, which include oral hygiene instructions, supragingival and subgingival scaling and root planing under local anaesthesia using ultrasonic instruments and hand instruments as needed. Patients were recalled after 3 months during which clinical parameters were recorded

SAMPLE PREPARAION

Collect fresh blood by venepuncture and 3 ml of blood was transferred to heparinised vacutainers and remaining 3 ml blood was transferred into clot activator tube **(Colour Plate – 4).** This clot activator tube was centrifuged at 3000 rpm for 3 minutes to separate serum **(Colour Plate – 5, 6).** Collected serum was transferred into ependorf tube. All the samples send on the same day to Genetika centre for advanced genetic studies, Pettah, Thiruvananthapuram, kerala.


Patients coming to the Department of Periodontics
HEALTHY CONTROLS
PATIENTS WITH CHRONIC PERIODONTITIS
PATIENTS WITH CHRONIC PERIODONTITIS, TYPE 2 DIABETES
Blood Sample Taken
LAB PARAMETERS
Micronuclei frequency
Malondialdehyde level
Hs – CRP level
HbA1C level
RBS
Total cholesterol
Triglyerides
PERIODONTAL PARAMETERS
Plaque Index (Silness and Loe , 1964)
Gingival Index (Loe and Silness, 1963)
Probing pocket depth
Clinical Attachment Level (CAL)
SCALING AND ROOT PLANING DONE
AFTER THREE MONTHS
LAB PARAMETERS
Micronuclei frequency
Malondialdehyde level
Hs – CRP level
HbA1C level
RBS
Total cholesterol
Triglycerides
PERIODONTAL PARAMETERS
Plaque Index (Silness and Loe , 1964)
Gingival Index (Loe and Silness, 1963)
Probing pocket depth
Clinical Attachment Level (CAL)

Protocol for CBMN assay

Collect fresh blood by venepuncture and transfer to heparinised vacutainers and the collected blood samples are send on the same day to Genetika centre for advanced genetic studies, Trivandrum.

1. Isolate lymphocytes on lymphoprep (pharmacia) gradients as follows: Add 2ml of lymphoprep to a 10ml centrifuge tube and carefully overlay 4ml of diluted blood sample **(Colour Plate – 8).**

2. Centrifuge at 1000rpm for 10min.

3. Drawn off the lymphocyte layer using a sterile Pasteur pipette and transfer to a 10ml tube.

4. Suspend the cell pellet in RPMI 1640 medium and centrifuge for 10 minutes.

5. Remove the supernatant and repeat step 5.

6. Culture the lymphocytes in sterile bottles using RPMI 1640 medium containing 15% foetal calf serum. Lymphocytes stimulate to divide with phytohaemagglutinin (PHA) and incubate for 72 hours at 37ºC **(Colour Plate – 9).**

7. 44 hr after PHA stimulation, add cytochalasin-B to the cultures to give a final concentration of 4.5µg/ml **(Colour Plate – 10).**

8. 28 hr after addition of cytochalasin –B, transfer the whole contents into a sterile centrifuge tube and centrifuge for 10 minutes; remove the supernatant, shack the pellet in a cyclomixer.

9. Add 10 ml of 0.075 M KCL solution to the cell button and kept at 37ºC for 10 minutes **(Colour Plate – 11).**

10. After this, add 2 drops of freshly prepared fixative (Methanol: Acetic acid) in the ratio 3:1.Again centrifuge at 1000 rpm for 10 minutes.

11. Remove the supernatant and mix the cell button in a cyclomixer and add 10 ml of freshly prepared fixative and centrifuge at 1000 rpm for 10 minutes.

12. Repeat this process until the supernatant becomes clear and the cell button becomes white.

13. From the cell button, prepare cell suspension.

14. Drop the cell suspension drop by drop on to pre cleaned, labeled, and chilled slides **(Colour Plate – 12).**

15. Air-dry the slides for 10 min, then fix in absolute methanol for 10 min and stain with May-Grunwald Giemsa for 10 minutes.

16. Code the slides before scoring and examine at 100X magnification **(Colour Plate – 13).** The number of MN in no less than 1000 binucleated cells should be scored and the distribution of MN among binucleated cells should be recorded **(Colour Plate – 14).**

CRITERIA FOR IDENTIFYING BINUCLEATED CYTOKINESIS BLOCK CELLS

The cytokinesis blocked cells scored for micronucleus frequency have to satisfy the following criteria:

- Cells should have two nuclei of approximately equal size;
- The 2 nuclei may be attached by a fine nucleoplasmic bridge; and
- The 2 nuclei may overlap slightly or touch each other at the edges.
- Cells should not contain more than 6 micronuclei.

CRITERIA FOR IDENTIFYING MICRONUCLEI

Micronuclei are morphologically identical to, but smaller than normal nuclei. They also have the following characteristics.

- Diameter between 1/6 and 1/3 that of the main nuclei.
- They are non-refractile.
- They are not linked to the main nuclei via a nucleoplasmic bridge.
- Micronuclei may sometimes overlap the boundaries of the main nuclei.

PREPARATION OF REAGENTS

Phytohaemagglutinin (PHA-M: 5mg stock):-

Dissolve 5mg stock of PHA-M in 5ml of distilled water.

Hypotonic KCl solution (0.075M):-

Dissolve 5.6gm of KCl (99.9%), (MW-74.55) in 1000ml of distilled water.

Fixative [Methanol and Glacial Acetic acid in the ratio 3:1 (v/v)]:-

Mix three parts of absolute methanol (99.8%) (32.04g/mol) and one part of glacial acetic acid (99.7%) (MW-60.05). Methanol acts as a fixative and acetic acid as the scavenger. Better morphological fixation occurs at low temperatures.

Cytochalasin-B: - Dissolve 1mg Cytochalasin-B in 1ml of dimethyl sulpoxide and store in freezer.

PREPARATION OF MEDIA

1) RPMI 1640 medium

1. Suspend the content of one unit vial in 950 ml of sterililized tissue culture grade water at room temperature with constant, gentle stirring until the medium is completely dissolved. Rinse the container with tissue culture grade water to remove all traces of powder and add to the above solution. Do not heat the water.

2. Add the required amount of sodium bicarbonate (2.25gm/l) as per the requirement and stir until dissolved.

3. Adjust the pH to 0.2-0.3 pH unit below the desired pH (7.4) using 1N Hydrochloric acid or Sodium Hydroxide since the pH tends to rise during the filtration.

4. Aseptically add sterile supplements (100µgm/ml Benzyl penicillin, 100units/ml streptomycin, 50µgm/ml Amphoterecin B, 15% foetal bovine serum, etc). Make up the final volume to 1000 ml with tissue culture grade water.

5. Sterilize the medium immediately by filtering through a sterile membrane filter with porosity of 0.2 micron or less using positive pressure to minimize the loss of carbon dioxide and dispensed the desired amount of sterile medium in to sterile containers. Store at 2-6°C till use.

Malondialdehyde (MDA) Malondialdehyde in serum is separated and determined as conjugate with Thiobarbituric acid (TBA). Serum proteins were precipitated by Trichloroacetic acid (TCA) and then removed by centrifugation. The MDA – TBA complex was measured at 540 nm[11].

Reagents

- Reagent A- 15% TCA in 100ml 0.25N Hydrochloric acid
- Reagent B- 0.375% TBA in 100ml 0.25N Hydrochloric acid
- Mix reagent A & B well and keep it in 2-8 ºC.

Protocol

1. Collect venous blood and transferred to a free tube, then allow clotting.
2. Centrifuged at 3000 rpm for 5 minutes & the serum are then removed to another plain tube & store at -2 ºC until the time of analysis.
3. Add 1ml of serum into 2ml of reagent (TCA+TBA+HCl).

4. The tubes were mixed well & incubated in boiling bath for 15 minutes.

5. Allow cooling, and then the tubes are left to stand at room temperature for 20 minutes.

6. Centrifuge at 2000 rpm for 15 minutes & then the supernatant layer was read at 540 nm **(Colour Plate – 7).**

The concentration of MDA (nmol/ml) was calculated by using the following formula:

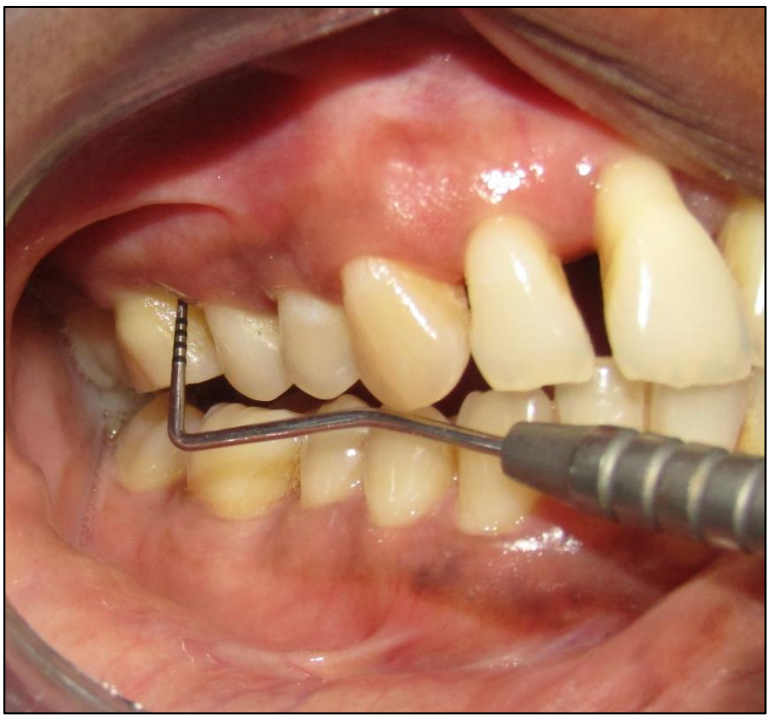
$$\text{Concentration of the test} = \frac{Abs(test)\text{x}10}{1.56}$$

Reference range = 0.7 – 1.9 U/l

High sensitive C Reactive Proteins or hs-CRP (Turbidometric Immunoassay)

Principle: The hs-CRP is a turbidmetric immunoassay for ultrasensitive determination of C-reactive protein in human serum. It is based on the principle of agglutination reaction. The test specimen is mixed with latex reagent and activation buffer and allowed to react **(Colour Plate – 15).** The presence of CRP in the test specimen results in the formation of insoluble complex producing turbidity, which is measured at a wavelength between 505-578 nm. The increase in turbidity corresponds to the concentration of CRP in the test specimen[17, 18] **(Colour Plate – 16).**

Colour Plates



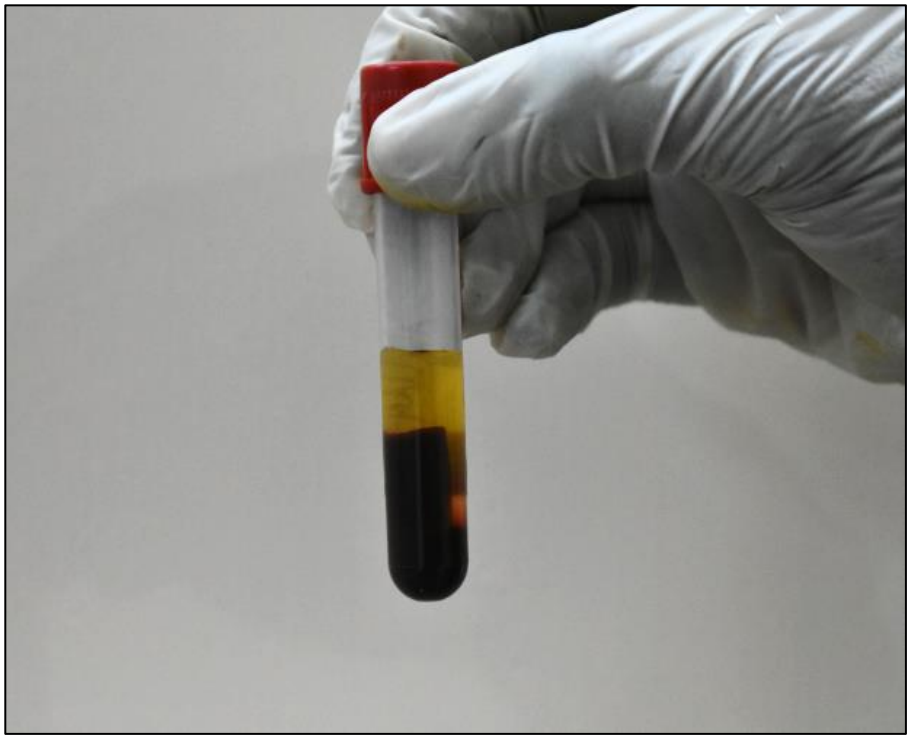
Colour plate 1 – Recording clinical parameters

Colour plate 2 – Armamentarium for non-surgical periodontal therapy

Colour plate 3 – Blood collection

Colour plate 4 – Vacutainer (BD) and Clot activator tube

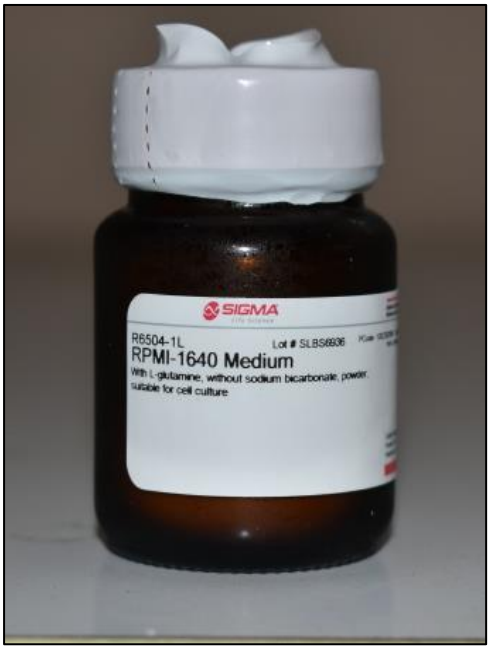
Colour plate 5 – REMI Laboratory centrifuge

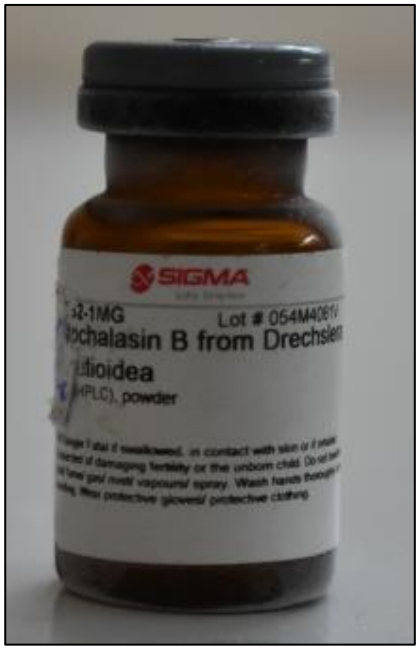


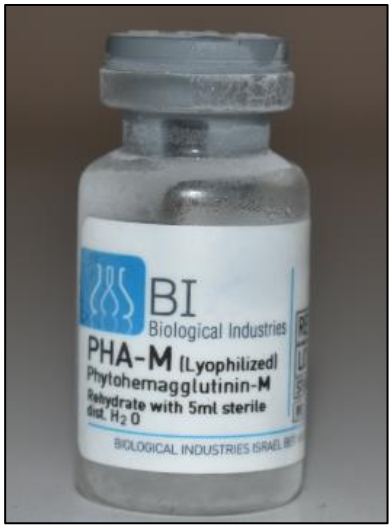
Colour plate 6 – Serum separation

Colour plate 7 – Semi auto analyser (MISPA – NEO) for MDA evaluation

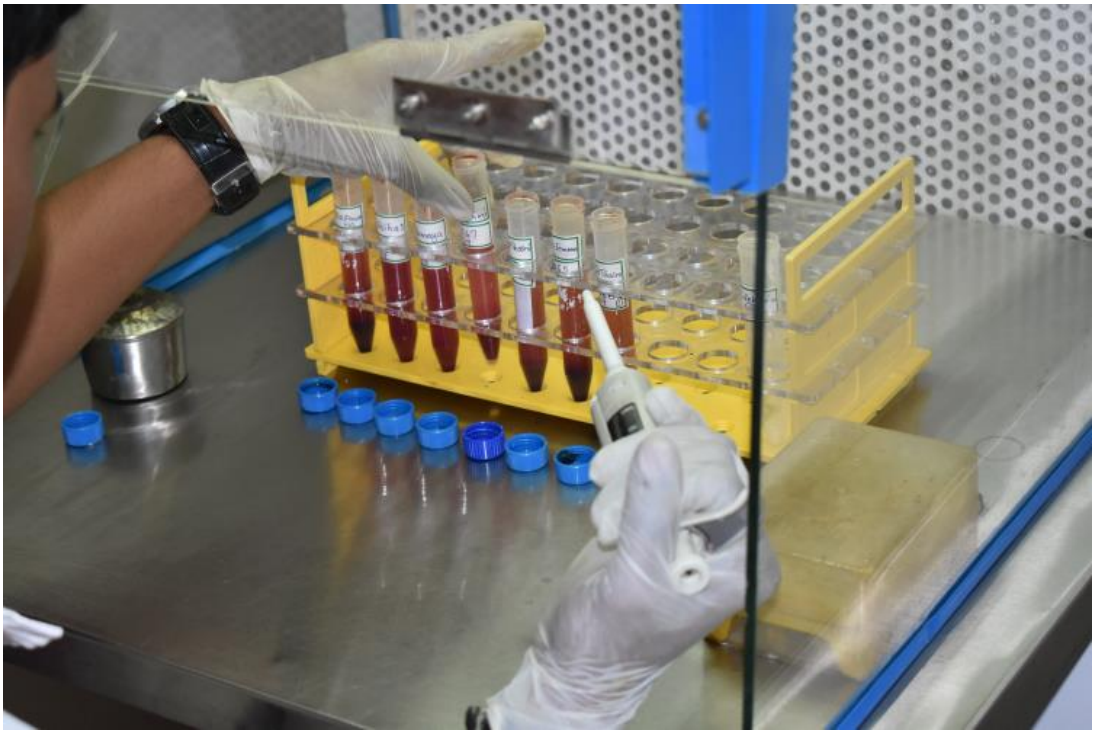
Colour plate 8 – Laminar air flow chamber





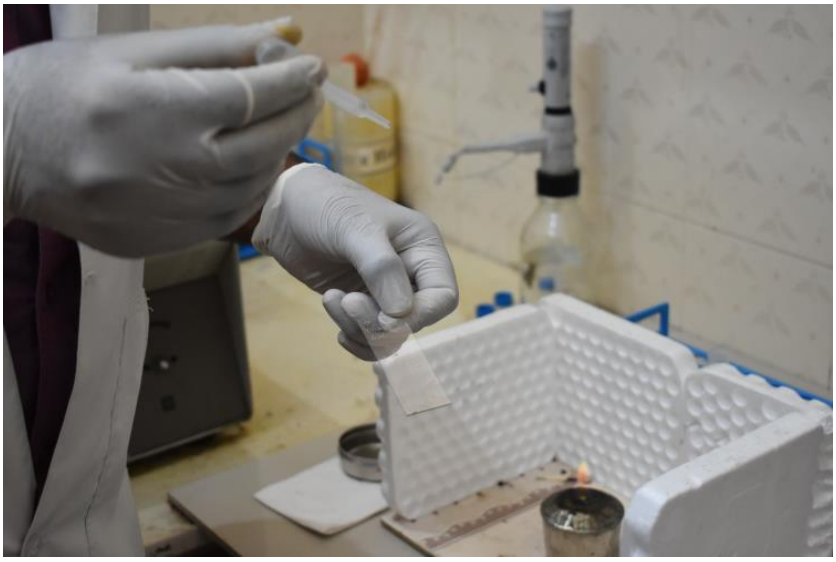


Colour plate 9 – Reagents for CBMN assay – RPMI-1640 (SIGMA), Cytochalasin B (SIGMA) and Phytohemagglutinin – M (BI)



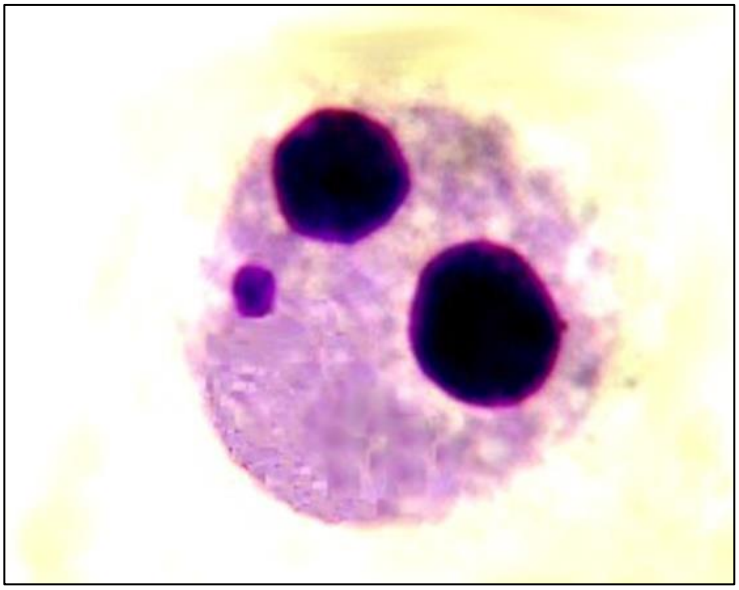
Colour plate 10 – Cell culturing

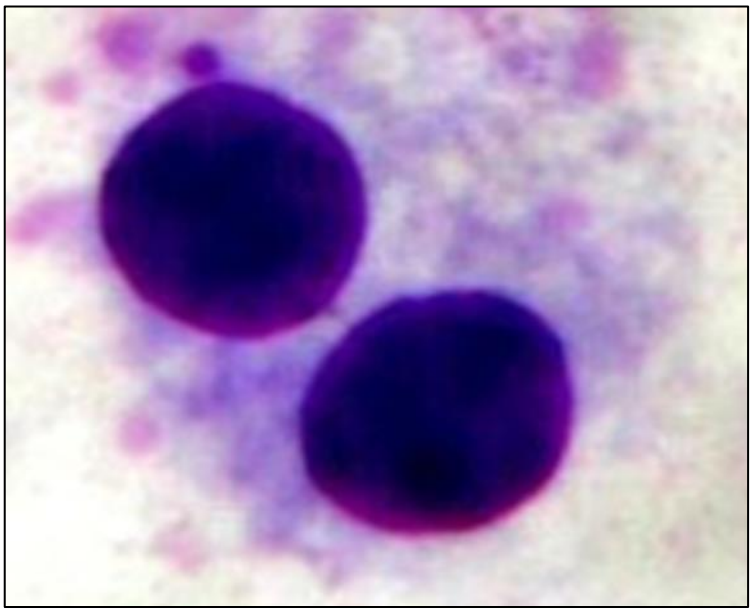
Colour plate 11 – Microbiological incubator (LABLINE)



Colour plate 12 – Cell harvesting

Colour plate 13 – Laboratory microscope (OLYMPUS CX31)





Colour plate 14 – Cytokinesis-block binucleated cells with Micronuclei

Colour plate 15 – Hs-CRP kit (EURO DIAGNOSTIC SYSTEMS)

Colour plate 16 – Semi auto analyser for Hs-CRP detection

Results & Observation

STATISTICAL ANALYSIS

In this study we had three groups and parameters studied were PI, GI, PPD, CAL, Mean CBMN frequency, Serum MDA level, Serum Hs-CRP level, HbA1C, RBS, Total cholesterol and Triglycerides. The data was analysed by SPSS software (version 22.0). For describing these data we used percentage, mean value, SD, median depending on the variable type. Comparison between three group's chronic periodontitis patients with well controlled type 2 DM, Chronic periodontitis patients, and healthy controls were done by using Chi square test, Parametric one way ANOVA and non-parametric one way ANOVA, whichever is appropriate. The results were presented in five different headings,

1. Baseline Demographic, Metabolic, Periodontal and Laboratory data of the participants [Mean (SD)]
2. Three Months Post-Operative Metabolic, and Periodontal data of the participants [Mean (SD)]
3. Changes in Probing Pocket Depth and Clinical Attachment Level at Baseline and after 3 Months (%)
4. Comparison of Periodontal and Laboratory data at baseline and 3 months after treatment [Mean (SD)]
5. Differences in Metabolic, and Periodontal data of the participants at baseline and after 3 months [Mean (SD)]

RESULTS AND OBSERVATIONS

TABLE 1: Baseline Demographic, Metabolic, Periodontal and Laboratory data of the participants [Mean (SD)]

PARAMETERS	GROUP 1	GROUP 2	GROUP 3	P VALUE
AGE (YEARS)	48.425 ± 6.9	36.926 ± 5.5	32.7 ± 2.5	0.000
GENDER M/F	22/6	22/5	22/6	0.954
PLAQUE INDEX (PI)	1.6 ± 0.22	1.61 ± 0.25	1.32 ± 0.18	0.00
GINGIVAL INDEX (GI)	1.8 ± 0.26	1.74 ± 0.32	1.31 ± 0.28	0.00
MEAN PPD (mm)	4.49 ± 0.37	3.86 ± 0.26	2.5 ± 0.20	0.00
MEAN CAL (mm)	4.80 ± 0.37	4.29 ± 0.41	2.5 ± 0.20	0.00
RBS (mg/dl)	146.6 ± 22.7	94.29 ± 8.2	90.3 ± 12.3	0.00
CBMN FREQUENCY (out of 1000 bi-nucleated cells)	13.20 ± 0.53	12.11 ± 0.43	9.84 ± 0.49	0.00
MDA LEVELS (U/L)	3.41 ± 0.29	2.73 ± 0.47	1.14 ± 0.33	0.00
Hs-CRP(mg/L)	8.77 ± 0.54	6.9 ± 0.51	1.21 ± 0.33	0.00
HbA1C (%)	6.48 ± 0.33	4.8 ± 0.56	3.5 ± 0.36	0.00
TOTAL CHOLESTEROL (mg/dl)	240.4 ± 22.1	167.4 ± 24.4	153.3 ± 25.7	0.00
TRIGLYCERIDES (mg/dl)	256.3 ± 57.41	114.1 ± 23.01	112.9 ± 13.5	0.00

Total of 83 participants were selected for this study of which 75 participants completed the study. Baseline demographic, metabolic, Periodontal and laboratory data are shown in Table 1. Twenty eight Chronic periodontitis patients with well controlled type 2 DM (Mean age 48.4 $\pm$ 6.9), Twenty seven Chronic periodontitis patients (Mean age 36.926 $\pm$ 5.5) and Twenty eight Healthy controls (Mean age 32.7 $\pm$ 2.5) were enrolled in this study. All of the clinical as well as the laboratory parameters were significantly higher in the group 1 and group 2 compared to group 3 at baseline.

TABLE 2: Three Months Post-Operative Metabolic, and Periodontal data of the participants [Mean (SD)]

PARAMETERS	GROUP 1	GROUP 2	GROUP 3	P VALUE
PLAQUE INDEX (PI)	0.34 ± 0.13	0.32 ± 0.14	0.27 ± 0.06	0.13
GINGIVAL INDEX (GI)	0.41 ± 0.19	0.41 ± 0.19	0.36 ± 0.39	0.017
MEAN PPD (mm)	2.59 ± 0.29	2.4 ± 0.19	2.5 ± 0.19	0.18
MEAN CAL (mm)	2.97 ± 0.30	2.76 ± 0.20	2.5 ± 0.19	0.00
RBS (mg/dl)	131.3 ± 20.4	91.12 ± 9.44	87.2 ± 9.4	0.00
CBMN FREQUENCY (out of 1000 bi-nucleated cells)	12.80 ± 0.46	11.87 ± 0.50	9.6 ± 0.45	0.00
MDA LEVELS (U/L)	2.5 ± 0.58	2.35 ± 0.49	1.01 ± 0.26	0.00
Hs-CRP(mg/L)	5.8 ± 1.32	4.2 ± 1.08	1.13 ± 0.31	0.00
HbA1C (%)	6.07 ± 0.30	4.65 ± 0.52	3.50 ± 0.33	0.00
TOTAL CHOLESTEROL (mg/dl)	210.6 ± 28.3	167.4 ± 20.4	145.1 ± 24.6	0.00
TRIGLYCERIDES (mg/dl)	225.08 ± 45.94	111.7 ± 20.7	108.2 ± 13.9	0.00

Table 2 shows the changes in the periodontal parameters after three months of non-surgical periodontal therapy. PI, GI, mean PPD shows no statistical difference (p Value >0.05) in between groups at 3 months post operatively. All other

parameters like Mean CAL, RBS, Mean CBMN frequency, MDA, Hs-CRP, Total cholesterol, Triglycerides, and HbA1C values are statistically significant in between 3 groups. (p Value <0.05)

Table 3: Changes in Probing Pocket Depth and Clinical Attachment Level at Baseline and after 3 Months (%)

PARAMETERS	**GROUP 1** **BASELINE** **POST OP**		**GROUP 2** **BASELINE** **POST OP**		**GROUP 3** **BASELINE** **POST OP**	
PPD ≤3mm (%)	50.5	92.29	56.5	97.6	92.08	96.60
PPD 4-6 mm (%)	42.35	6.27	40.5	2.35	7.9	3.39
PPD ≥ 7 mm (%)	7.1	1.42	2.94	0.0	0.0	0.0
CAL <3mm (%)	51.68	87.13	52.70	95.41	91.16	95.3
CAL 4-6 mm (%)	39.45	8.78	41.42	4.14	8.83	4.62
CAL ≥ 7 mm (%)	8.86	4.08	5.86	0.43	0.0	0.0

Table 3 given are changes in PPD (%), CAL (%) at baseline and after 3 months. The percentage of shallow sites (PPD ≤3mm) increased significantly in all the groups but more so in group 1 and group 2 than group 3, whereas the percentage of sites with PPD of 4-6mm and ≥ 7 mm decreased significantly in group 1 and 2 but more reduction occurred in group 2 than group 1. Similarly CAL % of shallow sites (≤3mm) increased significantly in all the groups but more so in group 1 and group 2 than group 3, whereas the percentage of sites with CAL of 4-6mm and ≥ 7 mm decreased significantly in group 1 and 2 but more reduction occurred in group 2 than

group 1 suggesting that more clinical attachment gain occurred in group 2 than group 1.

TABLE 4: Comparison of Periodontal and Laboratory data at baseline and 3 months after treatment [Mean (SD)]

PARAMETERS		GROUP 1	GROUP 2	GROUP 3	P VALUE
Mean PI Score	Baseline	1.6 ± 0.22	1.61 ± 0.25	1.32 ± 0.18	0.00
	Post op	0.34 ± 0.13	0.32 ± 0.14	0.27 ± 0.06	0.13
	p Value	0.00	0.00	0.00	
Mean GI score	Baseline	1.8 ± 0.26	1.74 ± 0.32	1.31 ± 0.28	0.00
	Post op	0.41 ± 0.19	0.41 ± 0.19	0.36 ± 0.39	0.017
	p Value	0.00	0.00	0.00	
Mean PPD (mm)	Baseline	4.49 ± 0.37	3.86 ± 0.26	2.5 ± 0.20	0.00
	Post op	2.59 ± 0.29	2.4 ± 0.19	2.5 ± 0.19	0.18
	p Value	0.00	0.00	0.53	
Mean CAL (mm)	Baseline	4.80 ± 0.37	4.29 ± 0.41	2.5 ± 0.20	0.00
	Post op	2.97 ± 0.30	2.76 ± 0.20	2.5 ± 0.19	0.00
	p Value	0.00	0.00	0.662	
RBS (mg/dl)	Baseline	146.6 ± 22.7	94.29 ± 8.2	90.3 ± 12.3	0.00
	Post op	131.3 ± 20.4	91.12 ± 9.44	87.2 ± 9.4	0.00
	p Value	0.00	0.145	0.016	
HbA1c (%)	Baseline	6.48 ± 0.33	4.8 ± 0.56	3.5 ± 0.36	0.00
	Post op	6.07 ± 0.30	4.65 ± 0.52	3.50 ± 0.33	0.00
	p Value	0.00	0.00	0.49	
Total Cholesterol (mg/dl)	Baseline	240.4 ± 22.1	167.4 ± 24.4	153.3 ± 25.7	0.00
	Post op	210.6 ± 28.3	167.4 ± 20.4	145.1 ± 24.6	0.00
	p Value	0.00	0.231	0.00	
Triglycerides (mg/dl)	Baseline	256.3 ± 57.41	114.1 ± 23.01	112.9 ± 13.5	0.00
	Post op	225.08 ± 45.9	111.7 ± 20.7	108.2 ± 13.9	0.00
	p Value	0.00	0.00	0.002	
CBMN frequency (out of 1000 bi-nucleated cells)	Baseline	13.20 ± 0.53	12.11 ± 0.43	9.84 ± 0.49	0.00
	Post op	12.80 ± 0.46	11.87 ± 0.50	9.6 ± 0.45	0.00
	p Value	0.00	0.00	0.03	
MDA (U/L)	Baseline	3.41 ± 0.29	2.73 ± 0.47	1.14 ± 0.33	0.00
	Post op	2.5 ± 0.58	2.35 ± 0.49	1.01 ± 0.26	0.00
	p Value	0.00	0.00	0.01	
Hs-CRP (mg/L)	Baseline	8.77 ± 0.54	6.9 ± 0.51	1.21 ± 0.33	0.00
	Post op	5.8 ± 1.32	4.2 ± 1.08	1.13 ± 0.31	0.00
	p Value	0.00	0.00	0.00	

Table 4 shows comparison of clinical and laboratory data at baseline and after 3 months. Mean PI score at baseline was 1.6 ± 0.22 in group 1, 1.61 ± 0.25 in group 2 and 1.32 ± 0.18 in group. This declined to 0.34 ± 0.13 in group 1, 0.32 ± 0.14, and 0.27 ± 0.06 in group 3. Intergroup comparison of post-operative PI score is statistically not significant. Similarly GI score also decreased dramatically from baseline to 3 months. Mean GI score at baseline was 1.8 ± 0.26 in group 1, 1.74 ± 0.32 in group 2 and 1.31 ± 0.28 in group. This declined to 0.41 ± 0.19 in group 1, 0.41 ± 0.19 in group 2, and 0.36 ± 0.39 in group 3. Other clinical parameters like Mean PPD and Mean CAL also decreased significantly after 3 months. Mean PPD at baseline was 4.49 ± 0.37 in CP with DM group, 3.86 ± 0.26 in CP group and 2.5 ± 0.20 in control group. This value reduced to 2.59 ± 0.29 in CP with DM group, 2.4 ± 0.19 in CP group, and 2.5 ± 0.19 in control group. Similarly Mean CAL at baseline was 4.80 ± 0.37 in CP with DM group, 4.29 ± 0.41 in CP group and 2.5 ± 0.20 in control group. This value reduced to 2.97 ± 0.30 in CP with DM group, 2.76 ± 0.20 in CP group, and 2.5 ± 0.19 in control group. Intra group comparison reveals that significant reduction of PPD and CAL in group 1 and group 2 (p Value <0.05) but not in group 3.

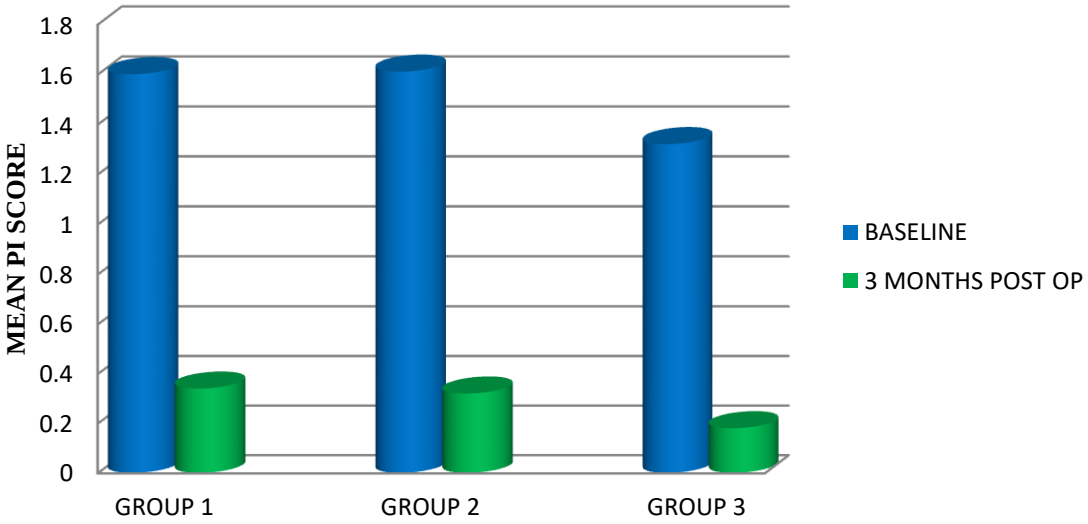
TABLE 5: Differences in Metabolic, and Periodontal data of the participants at baseline and after 3 months [Mean (SD)]

PARAMETERS	GROUP 1	GROUP 2	GROUP 3	P VALUE
PLAQUE INDEX (PI)	1.3 ± 0.2	1.2 ± 0.2	1.07 ± 0.2	0.001
GINGIVAL INDEX (GI)	1.4 ± 0.2	1.2 ± 0.2	1.0 ± 0.4	0.00
MEAN PPD (mm)	1.9 ± 0.4	1.3 ± 0.3	0.03 ± 0.2	0.00
MEAN CAL (mm)	1.8 ± 0.4	1.5 ± 0.5	0.02 ± 0.2	0.00
RBS (mg/dl)	16 ± 20.9	2.8 ± 9.2	2.8 ± 5.5	0.001
CBMN FREQUENCY (out of 1000 bi-nucleated cells)	0.38 ± 0.35	0.27 ± 0.21	0.15 ± 0.24	0.014
MDA LEVELS (U/L)	0.88 ± 0.59	0.39 ± 0.28	0.15± 0.16	0.00
Hs-CRP (mg/L)	2.9 ± 1.36	2.72 ± 0.94	0.06 ± 0.09	0.00
HbA1C (%)	0.41 ± 0.13	0.24 ± 0.22	0.02 ± 0.01	0.00
TOTAL CHOLESTEROL (mg/dl)	32.6 ± 35.5	3.0 ± 12.19	6.4 ± 5.4	0.00
TRIGLYCERIDES (mg/dl)	25.6 ± 26.3	4.4 ± 3.73	3.6 ± 5.4	0.00

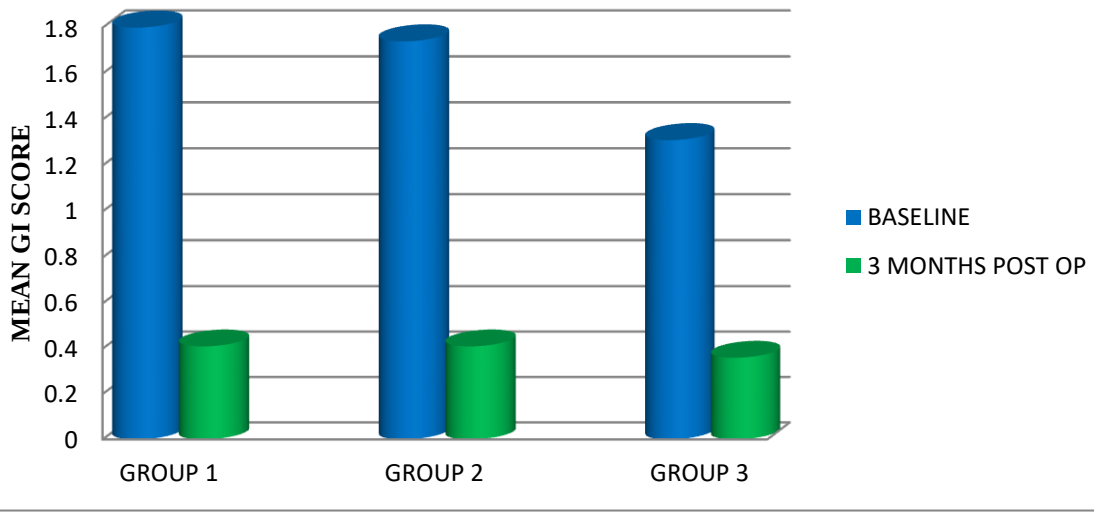
Table 5 shows the amount of reduction occurred in Periodontal as well as laboratory parameters from baseline to 3 months post operatively. Mean PI and Mean GI scores reduced significantly in all the three groups. Mean PI score reduction is 1.3 $\pm$ 0.2 in CP with DM group, 1.2 $\pm$ 0.2 in CP group and 1.07 $\pm$ 0.2 in control group. PI score reduction occurs more in group 1 and group 2 than group 3. Similarly GI score reduction is 1.4 $\pm$ 0.2 in CP with DM group, 1.2 $\pm$ 0.2 in CP group and 1.0 $\pm$ 0.4 in control group. Intergroup comparison reveals Mean PPD and Mean CAL reduction occurs more in CP with DM and CP group than in control group. Random blood sugar, Total Cholesterol and Triglyceride levels reduced significantly in all the groups. But more reduction occurs in CP with DM group than CP and control group. Reduction of Mean CBMN frequency, MDA and Hs-CRP occurs more in CP with DM followed by CP group and finally least reduction occurs in control group.

Graphs

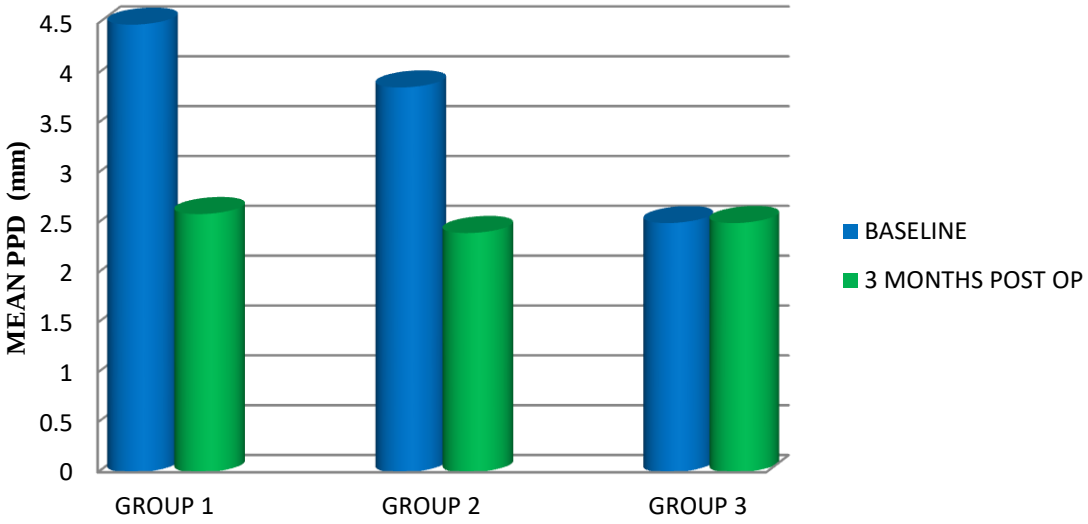
GRAPH 1: COMPARISON OF MEAN PI SCORE OF DIFFERENT GROUPS AT BASELNE AND AFTER 3 MONTHS



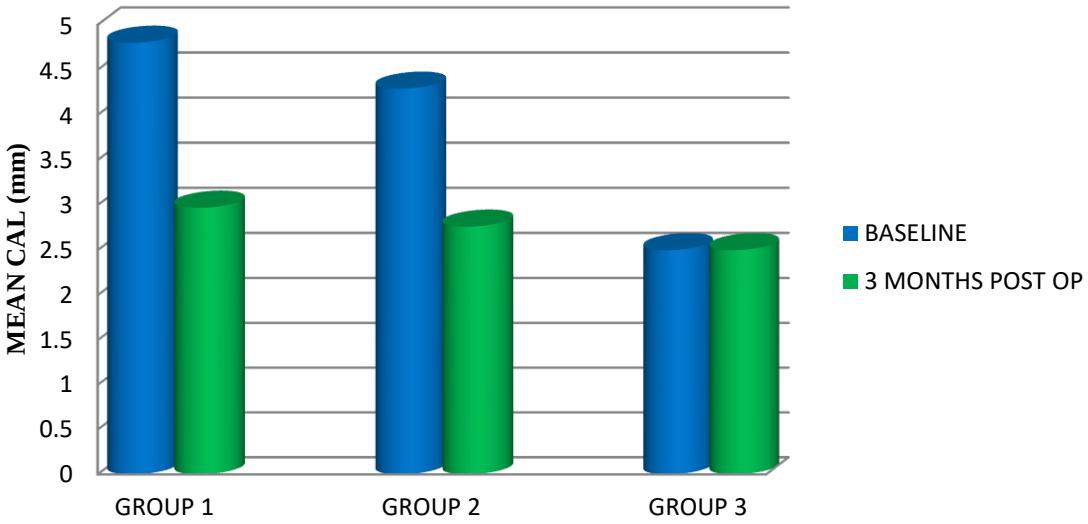
GRAPH 2: COMPARISON OF MEAN GI SCORE OF DIFFERENT GROUPS AT BASELINE AND AFTER 3 MONTHS



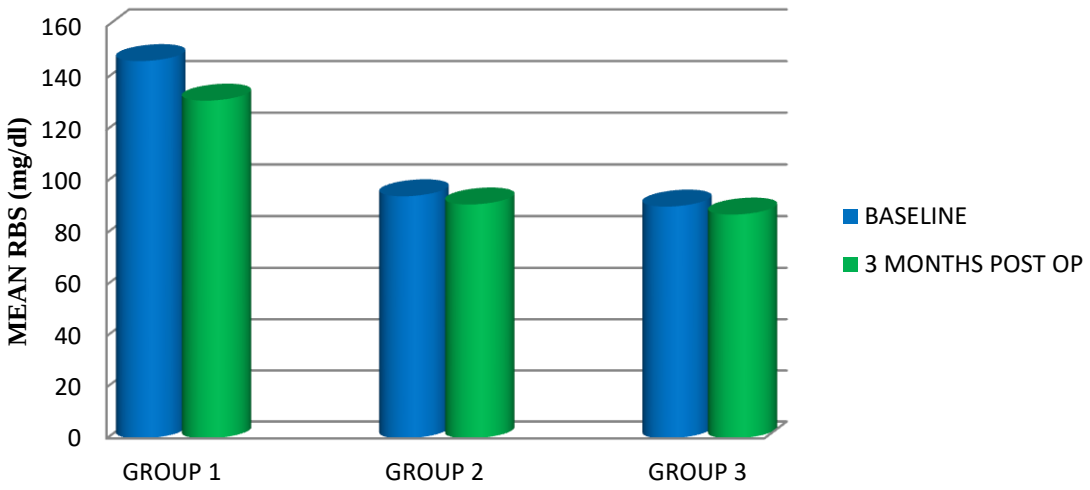
GRAPH 3: COMPARISON OF MEAN PPD OF DIFFERENT GROUPS AT BASELINE AND AFTER 3 MONTHS



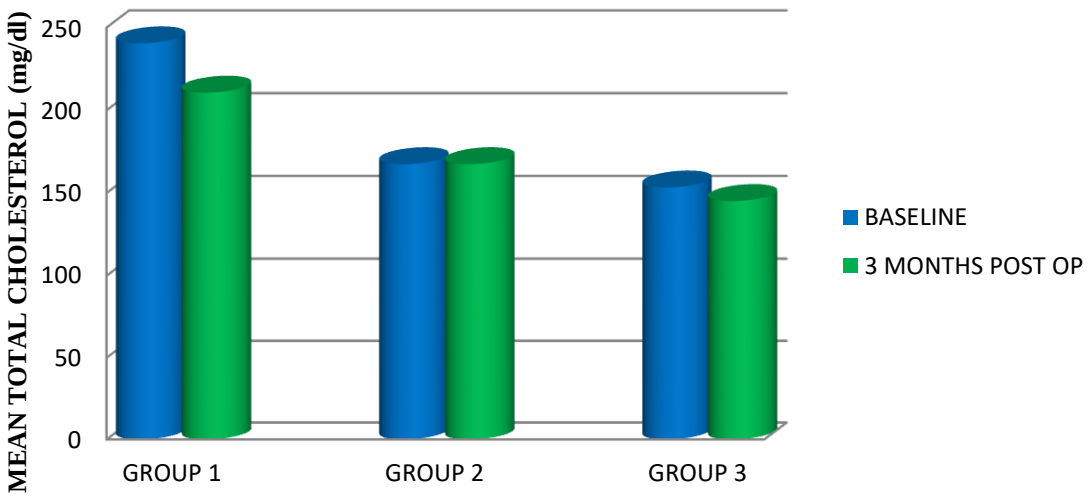
GRAPH 4: COMPARISON OF MEAN CAL OF DIFFERENT GROUPS AT BASELINE AND AFTER 3 MONTHS



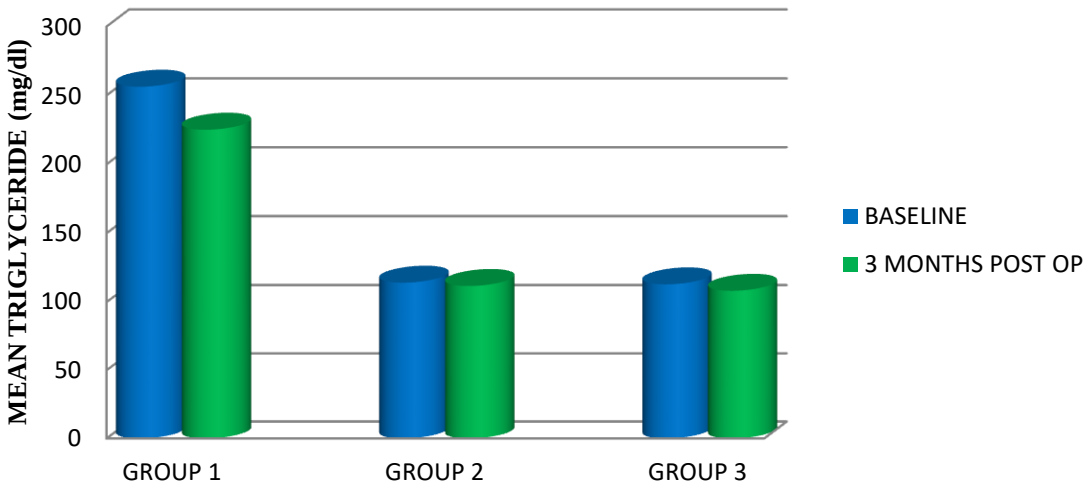
GRAPH 5: COMPARISON OF MEAN RBS VALUE OF DIFFERENT GROUPS AT BASELINE AND AFTER 3 MONTHS



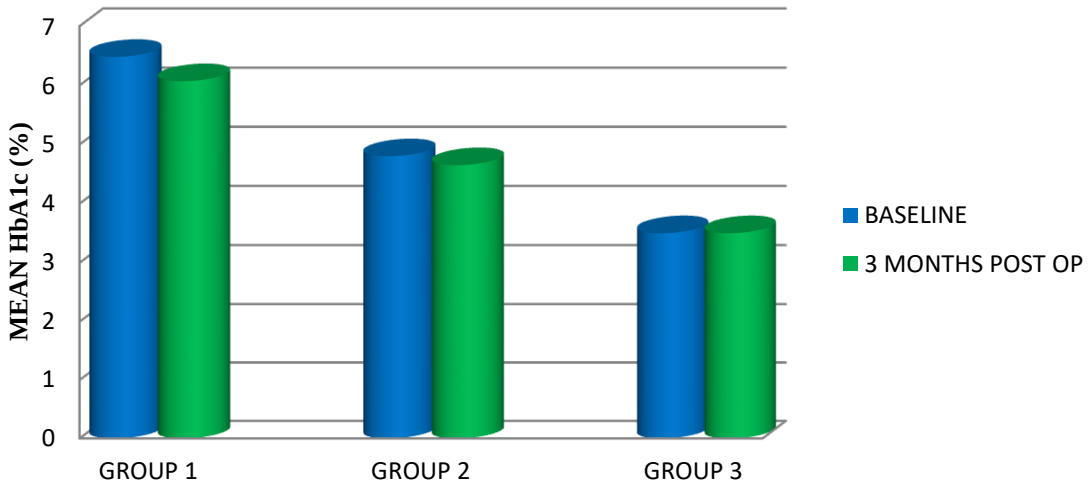
GRAPH 6: COMPARISON OF MEAN TOTAL CHOLESTEROL VALUE OF DIFFERENT GROUPS AT BASELINE AND AFTER 3 MONTHS



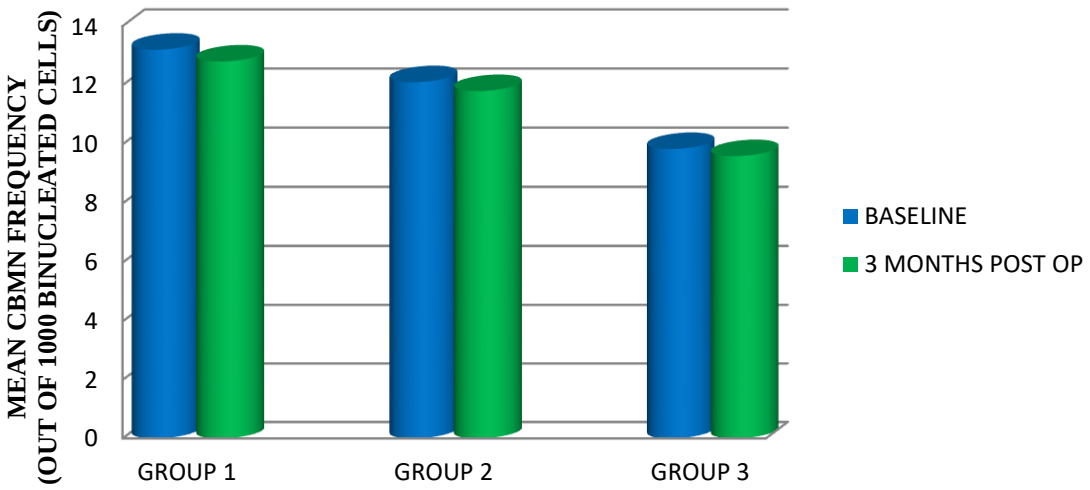
GRAPH 7: COMPARISON OF MEAN TRIGLYCERIDE VALUE OF DIFFERENT GROUPS AT BASELINE AND AFTER 3 MONTHS



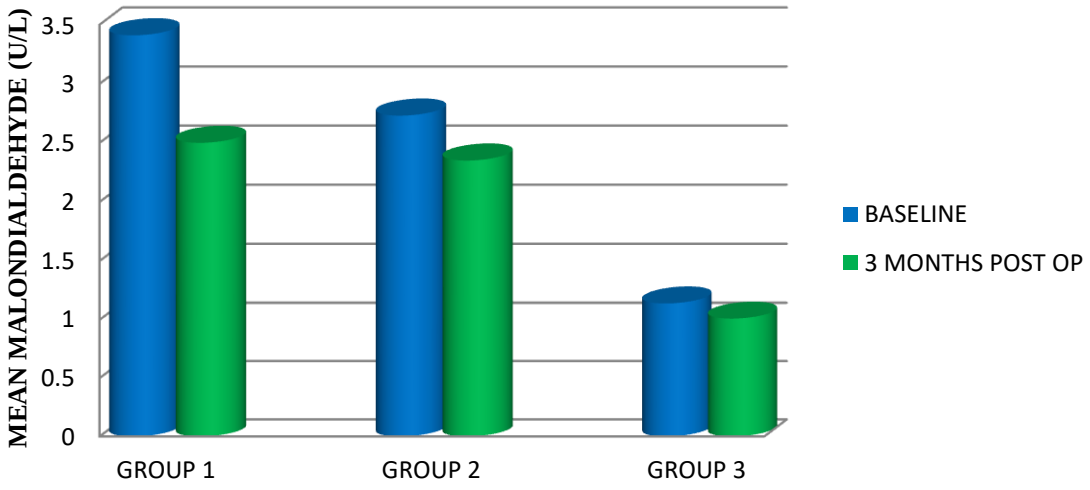
GRAPH 8: COMPARISON OF MEAN HbA1c VALUE OF DIFFERENT GROUPS AT BASELINE AND AFTER 3 MONTHS



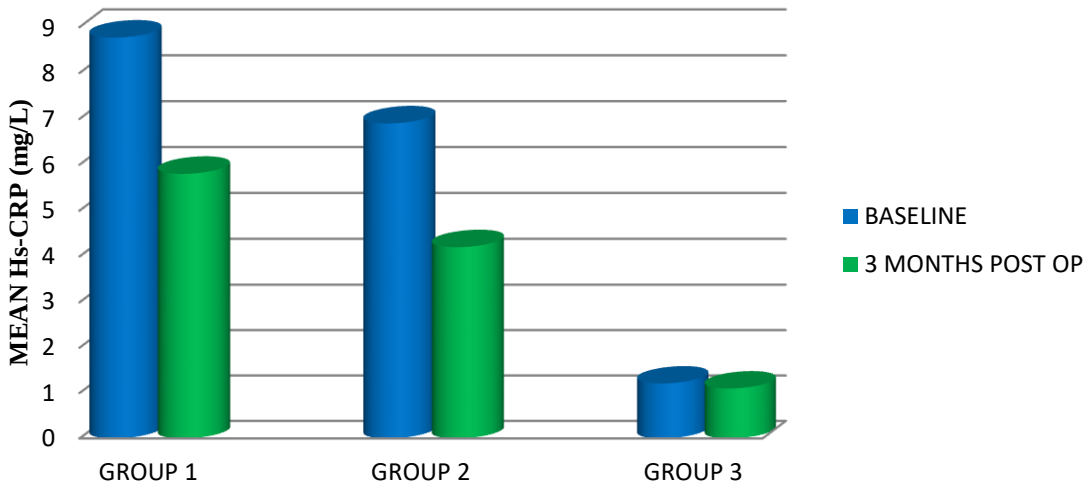
GRAPH 9: COMPARISON OF MEAN CBMN FREQUENCY OF DIFFERENT GROUPS AT BASELINE AND AFTER 3 MONTHS



GRAPH 10: COMPARISON OF MEAN MALONDIALDEHYDE LEVELS OF DIFFERENT GROUPS AT BASELINE AND AFTER 3 MONTHS



GRAPH 11: COMPARISON OF MEAN Hs-CRP LEVELS OF DIFFERENT GROUPS AT BASELINE AND AFTER 3 MONTHS



Discussion

Periodontitis is a chronic infectious disease, which is usually initiated by an imbalance between the oral microbial community and host immuno-inflammatory response. The initial host response is the release of reactive oxygen species (ROS) via the metabolic process of respiratory burst in the Polymorphonuclear leukocytes (PMNL's), macrophages and monocytes. Excessive production of these ROS results in oxidative stress in the body. Diabetes mellitus, for many years, has been recognized as an important risk factor for periodontal diseases and is associated with significantly higher prevalence and severity of periodontitis. In both the diseases there is increased ROS production. Among the ROS formed, H_2O_2 and OH radicals are potent oxidants that can affect nucleic acids and modify bases in the DNA, contributing to an increase in DNA damage and therefore Micronuclei formation.[12] Micronuclei were first described by Howell and Jolly in late 1800's and early 1900's as Feulgen positive nuclear bodies in human reticulocytes, representing chromosomes separated from the mitotic spindle[66].

The aim of the current study was to compare the micronuclei frequency in peripheral blood, serum Malondialdehyde level, and serum high sensitive C - reactive protein at baseline and 3 months after non-surgical periodontal therapy in chronic periodontitis patients with and without well controlled type 2 diabetes mellitus, and the patients with healthy periodontium.

In the present study total of 83 patients were selected at baseline. Twenty eight Chronic periodontitis patients with well controlled type 2 DM (Mean age 48.4 $\pm$ 6.9), Twenty seven Chronic periodontitis patients (Mean age 36.9 $\pm$ 5.5) and Twenty eight Healthy controls (Mean age 32.7 $\pm$ 2.5) were enrolled in this study. Of which 75 patients completed the three months protocol.

In the present study at baseline all the periodontal parameters like PI, GI, PPD, and CAL were elevated in group 1 and group 2 than group 3. There was no statistical difference in PI, GI at baseline among three groups. In terms of mean PPD and mean CAL was more in DM group compared to CP group. In other words chronic periodontitis with well controlled type 2 DM had more periodontal breakdown than in patients with chronic periodontitis alone. This shows that DM has additional effect on periodontium. 3 months after SRP all the clinical parameters are reduced significantly in all the groups. These findings were consistent with previous study by Kudva et al 2010 found out that highly significant reduction in the plaque index scores, Gingival index, and Probing pocket depth was seen in both the control and test groups from baseline to 3 months.[67] Similarly other studies also found out statistically significant reduction in plaque score, gingival index score, probing pocket depth and mean CAL after non-surgical periodontal treatment from baseline to 3 months.[68-69] The results of the present study demonstrated a positive correlation between PI, GI, probing depth and clinical attachment level with other laboratory parameters.

In the present study the 3-months interval was chosen because glucose in blood is irreversibly bound to haemoglobin. The glycated haemoglobin indicates the glucose status during at least half of the life of Red blood cells that is 30 to 90 days because the half-life of blood cells is 120 days.[70]

After treatment, there were improvements in all of the monitored clinical parameters. These improvements were reflected at the systemic level by alterations in serum inflammatory markers and as verified in previous studies, a reduction in HbA1c. Clinical trial by Agarwal et al 2016 provided evidence that elimination of

periodontal inflammation, significantly ($P < 0.05$) reduce the HbA1c level, thus improving diabetic metabolic control.[71] Similarly study by Aryal et al 2017 suggest that following periodontal therapy there was an improvement in glycemic control in individuals with type 2 DM when compared with a non-treatment control group.[44]

In our study HbA1c level reduced 0.41 $\pm$ 0.13 in CP with DM group. Recent systematic review and meta-analysis by Teshome et al 2017 concluded that mean HbA1c reduction after periodontal treatment was 0.51%.[42]

Similarly another systematic review by Corbella et al 2013 found out that the mean decrease of HbA1c after periodontal treatment was 0.38% after 3 months and 0.31% after 6 months[48]. Another study by Kanduluru et al 2014 stated that, three months after SRP 0.35% reduction of HbA1c occurs in chronic periodontitis patients with type 2 diabetes mellitus[18].

In the present study significant elevated levels of mean CBMN frequency in CP with DM group and CP alone group compared to control group. Consistent findings are also found by Rathod et al 2016[12], concluded that patients with chronic periodontitis and DM had increased CBMN frequency. Another study by Zamora-Perez et al 2015 concluded that micronuclei frequency was elevated in buccal mucosal cells of chronic periodontitis patients.[22] Results by Bastos-Aires et al 2013 indicated a 2.3-fold increase in MN basal levels in patients with moderate to severe periodontitis compared to the control patients ($P < 0.001$), suggesting that the periodontal status may affect MN reference levels.[23]

Shettigar et al 2012 found out that increased glycosylation seems to induce oxidative damage in the DNA of the diabetic patients, which manifests as an

increased micronuclei frequency[25]. Zuniga-Gonzalez et al 2007 demonstrated that either controlled (glycosylated haemoglobin levels >7%) or uncontrolled diabetic patients (glycosylated haemoglobin levels <7%) had 2-fold higher frequency of MNs in buccal mucosa samples than healthy subjects.[26]

In our study we used peripheral lymphocytes for assessing the micronuclei frequency instead of taking saliva or GCF or buccal mucosal cells because these sources have following disadvantages; (i) Saliva and GCF have storage difficulties, (ii) Systemic effects cannot be measured in these locally expressed fluids, (iii) Any radiation exposure to oral cavity can cause MN formation in buccal mucosal cells, which could lead to mis-interpretation of results, (iv) Very less quantity of GCF can be obtained.

Also in our study we selected the patient with well controlled type 2 DM (HbA1c value 6.5-7.0%) for group 1 rather than poorly controlled diabetics. Because uncontrolled DM causes more periodontal destruction, thus we cannot differentiate the reasons for the elevated levels of biomarkers. Also periodontal therapy is less effective in poorly controlled diabetic subjects and bad metabolic control could reveal a failure of pharmacological and non-pharmacological measures of disease control[72]. In our study we have not used any adjunctive antibiotic therapy other than scaling and root planing to all the participants. Because using systemic/local antibiotic therapy could affect the clinical results.

After three months of non-surgical periodontal therapy, mean CBMN values reduced statistically significant in all the three groups (p Value <0.05). To the best of our knowledge this is the first study which evaluated the effect of non-surgical

periodontal treatment on CBMN frequency in Diabetic chronic periodontitis patients.

Excessive production of ROS results in oxidative stress in the body. Reactive oxygen species (ROS) have a very short life (10^{-6}-10^{-9} seconds), so it is not easy to detect its presence. The ROS-mediated tissue destruction could be measured by the final product of lipid peroxidation (LPO), such as malondialdehyde (MDA).[1]

In our study mean MDA level was elevated in CP with DM group and CP alone group than control group. Among these CP with DM group showed increased MDA level than CP alone group. These results are consistent with previous studies suggesting that increased MDA levels in chronic periodontitis and DM[1, 30, 73, 74].

The main mechanism behind the elevation of MDA is the disturbance in anti-oxidant defense system due to overproduction of LPO products at inflammatory sites. This could be related to a higher level of oxidative stress in periodontitis and diabetes patients[1]. After three months of non-surgical periodontal therapy mean MDA levels were reduced in all the groups. This reduction was statistically significant (p Value <0.05).

Random blood sugar and total cholesterol also reduced in all the groups but the reduction was statistically significant in group 1 and group 3 not in group 2. Triglycerides also declined significantly in all the groups. Similarly, many other studies have reported that total cholesterol and triglyceride levels in patient with chronic periodontitis were significantly higher than those of the healthy subjects[75, 76]. Jaramillo et al 2013 stated that untreated periodontitis is associated to possible alterations of lipid metabolism[77].

Tendon et al[78] evaluated the effect of periodontal disease on the serum lipid levels and found that periodontal therapy resulted in significant decrease in the levels of serum total cholesterol, trigycerides and LDL-cholesterol.

They also stated that the underlying mechanism may be the local production of inflammatory cytokines and its effect on other systemic mediators leading to alterations in lipid metabolism, such as increased LDL and triglycerides, due to increased hepatic lipogenesis, lipolysis from adipose tissue, or reduced blood clearance[78].

In our study 3 months after periodontal treatment total cholesterol and triglyceride levels reduced significantly. This is because of the fact that non-surgical periodontal therapy reduces the systemic inflammatory burden.

C - reactive protein (CRP) is a plasma protein, pentameric in nature, which participates in systemic response to inflammation. Its homologoes are formed in vertebrates and many invertibrates. It has extremely high sensitivity but very poor specificity.[79] CRP is known to be produced primarily in the liver, synthesized by hepatocytes in response to inflammatory cytokines particularly IL-6. It reaches peak levels quickly in approximately 50 hours, and falls once the inflammatory stimulus is removed and has a half-life of 18 hours.[80]

All studies on periodontitis patients with diabetes clearly demonstrate that the inflammatory response in such patients is related to an increase in CRP[13]. In our study Hs-CRP levels were significantly elevated in group 1 and group 2 than group 3. Recent investigations emphasized the role of moderate elevation of CRP levels as a risk factor for cardiovascular diseases[14]. Mechanism behind the CRP increase in

our study is that the periodontitis and DM contributes to the elevation of systemic inflammation.

After 3 months of non-surgical periodontal therapy, mean Hs-CRP levels reduced significantly in all the groups (p Value <0.05). This is consistent with the previous studies, reporting that effective non-surgical periodontal treatment can reduce the serum Hs-CRP levels in chronic periodontitis patients with type 2 diabetes mellitus[15, 49]. Similarly a study by Marcaccini et al 2009 found out that, IL-6 and Hs-CRP concentrations decreased 3 months after non-surgical periodontal therapy in patients with periodontal disease[53].

Similarly systematic reviews by Demmer et al 2013, de Freites et al 2012, and Ioannidou et al 2006 also reported that there was a significant reduction of systemic CRP following periodontal treatment[81-83].

Increased Micronuclei frequency, MDA, and Hs-CRP levels were linked with many cardiovascular diseases. Andreassi et al 2011 stated that an elevated level of MDA indicates increase in production of oxygen free radicals suggesting the possible risk in atherogenesis, leading to coronary heart diseases[16]. HsCRP is a powerful marker of vascular risk in a wide range of populations and, as such, is a powerful research tool in assessing at-risk populations. Determination of these markers contributes to understanding the development of and prevention of CVS disorders[17]. Our study results demonstrate that chronic periodontitis patients with well controlled type 2 DM have increased risk of cardiovascular diseases at baseline. After three months of non-surgical periodontal therapy all the biomarker levels were reduced due to the elimination of inflammatory burden. This helps to reduce the

cardiovascular risk of the patients with chronic periodontitis and type 2 DM. Early detection of these biomarkers will help the clinician to plan the treatment accordingly to reduce the occurrence of cardiovascular events. The findings of the current study demonstrated that periodontal therapy brought about significant changes in the clinical as well as laboratory parameters of study subjects reinforcing the hypothesis that there is an inter-relationship between periodontitis, DM and cardiovascular diseases.

The present study has some of the limitations that need to be clearly discussed which include, the short duration of the follow up (3 months). Further longitudinal studies are necessary to rule out the perio-systemic association. Also, the current study was cross sectional in nature with a modest sample size and cannot be used to determine the causal relationship. Thus, cohort studies with large sample sizes should be conducted to obtain comprehensive information. The calibration of the examiner who performed clinical examination was not carried out. Thus, the effectiveness of this procedure might be biased.

Summary & Conclusion

Present study compares the MN frequency, serum MDA and serum Hs-CRP levels in chronic periodontitis patients with and without well controlled type 2 diabetes mellitus and healthy controls at baseline and 3 months after non-surgical periodontal therapy. All the clinical and laboratory parameters were elevated at baseline in all the groups. Elevated levels of these bio markers indicate the patient is under cardiovascular risk. After three months of non-surgical periodontal therapy all the biomarker levels were reduced due to the elimination of inflammatory burden. The findings of the current study demonstrated that periodontal therapy brought about significant changes in the clinical as well as laboratory parameters of patients with chronic periodontitis and well controlled type 2 diabetes mellitus. This helps to reduce the cardiovascular risk of the patients with chronic periodontitis and type 2 DM.

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Annexures

Genetika
Centre for Advanced Genetic Studies

October 5, 2017

CERTIFICATE

This is to certify that I have no objection for extending and using the available facilities of this centre for carrying out to do the research on the topic **"EFFECT OF NON SURGICAL PERIODONTAL THERAPY ON THE MICRONUCLEI FREQUENCY, SERUM MALONDIALDEHYDE AND SERUM HIGH SENSITIVE C-REACTIVE PROTEIN LEVELS IN CHRONIC PERIODONTITIS PATIENTS WITH AND WITHOUT WELL CONTROLLED TYPE II DIABETES MELLITUS"** by Dr. Blessing Emmanuel P, MDS second year student, Department of periodontics, Sree Mookambika Institute of Dental Sciences, Kulasekaram, under the guidance of Dr. Arun Sadasivan MDS.

Centre for Advanced Genetic Studies Genetika Trivandrum-695 024

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SREE MOOKAMBIKA INSTITUTE OF DENTAL SCIENCES

KULASEKHARAM, KANYAKUMARI DIST., TAMIL NADU, INDIA.

INSTITUTIONAL RESEARCH COMMITTEE

Certificate

This is to certify that the research project protocol, ***Ref no. 17/09/2017*** titled, ***"Effect of Non Surgical Periodontal Therapy on the Micronuclei Frequency, Serum Malondialdehyde and Serum High Sensitive C – Reactive Protein Levels in Chronic Periodontitis Patients with and without Well Controlled Type 2 Diabetes Mellitus"*** submitted by ***Dr. Blessing Emmanuel P, II MDS, Department of Periodontics"*** has been approved by the Institutional Research Committee at its meeting held on ***17th October 2017.***

Convener
Dr. T. Sreelal

Secretary
Dr. Pradeesh Sathyan

INSTITUTIONAL HUMAN ETHICS COMMITTEE

SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES,
KULASEKHARAM, TAMILNADU

Communication of Decision of the Institutional Human Ethics Committee(IHEC)

SMIMS/IHEC No: 2 / Protocol no: 36 / 2017

Protocol title: EFFECT OF NON SURGICAL PERIODONTAL THERAPY ON THE MICRONUCLEI FREQUENCY,SERUM MALONDIALDEHYDE AND SERUM HIGH SENSITIVE C – REACTIVE PROTEIN LEVELS IN CHRONIC PERIODONTITIS PATIENTS WITH AND WITHOUT WELL CONTROLED TYPE 2 DIABETES MELLITUS
Principal Investigator: Dr.Blessing Emmanuel.P
Name& Address of Institution: Department of Periodontics and Oral Implantology Sree Mookambika Institute of Dental Sciences ☑ New review ☐ Revised review ☐ Expedited review
Date of review (D/M/Y): 05-12-2017 Date of previous review , if revised application:
Decision of the IHEC: ☑ Recommended ☐ Recommended with suggestions ☐ Revision ☐ Rejected
Suggestions/ Reasons/ Remarks:
Recommended for a period of :One year

Please note*

- Inform IHEC immediately in case of any Adverse events and Serious adverse events.
- Inform IHEC in case of any change of study procedure, site and investigator
- This permission is only for period mentioned above. Annual report to be submitted to IHEC.
- Members of IHEC have right to monitor the trial with prior intimation.

SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES ★ KULASEKHARAM ★
IHEC
Date..6/12/17..

Reneegalgangadhar

Signature of Member Secretary (IHEC)

CONSENT FORM

PART 1 OF 2

INFORMATION FOR PARTICIPANTS OF THE STUDY

Dear Volunteers,

We welcome you and thank you for your keen interest in participation in this research project. Before you participate in this study, it is important for you to understand why this research is being carried out. This form will provide you all the relevant details of this research. It will explain the nature, the purpose, the benefits, the risks, the discomforts, the precautions and the information about how this project will be carried out. It is important that you read and understand the contents of the form

1. **Name of the Principal Investigator :**Dr. Blessing Emmanuel P
   Second Year Post Graduate student
   Department of Periodontics
   Sree Mookambika Institute of Dental Sciences,
   Kulasekharam

2. **Name of the Guide:** Dr. Arun Sadasivan MDS
   Professor
   Department of Periodontics
   Sree Mookambika Institute of Dental Sciences
   Kulasekharam, Kanyakumari District-629161

3. **Name of the Co-Guide:** Dr. Elizabeth Koshi MDS
   Professor and Head
   Department of Periodontics
   Sree Mookambika Institute of Dental Sciences
   Kanyakumari District-629161

4. **Institute:** Sree Mookambika Institute of Dental Sciences,
   V.P.M Hospital complex, Padanilam,
   Kulasekharam, Kanyakumari District – 629161
   Tamilnadu

5. **Title of the study:** "EFFECT OF NON SURGICAL PERIODONTAL THERAPY ON THE MICRONUCLEI FREQUENCY, SERUM MALONDIALDEHYDE AND SERUM HIGH SENSITIVE C – REACTIVE PROTEIN LEVELS IN CHRONIC PERIODONTITIS PATIENTS WITH AND WITHOUT WELL CONTROLLED TYPE 2 DIABETES MELLITUS"

6. Background information:

Periodontitis is a multifactorial disease which is mainly caused by pathogenic bacteria. Polymorphonuclear leukocytes (PMNs) are the primary mediators of host response against pathogens and they generate increased levels of reactive oxygen species (ROS). The ROS-mediated tissue destruction could be measured by the final product of lipid peroxidation (LPO), such as malondialdehyde (MDA). Type 2 diabetes mellitus is the most common form of DM One of the main consequences of this adverse action is the formation of oxidative stress.[2]The oxidative stress is intrinsically related to the pathogenesis of type 2 diabetes, dyslipidemia and PD, and it leads to DNA damage.

7. Aims and Objectives

The aim of the study is to evaluate the effect of non-surgical periodontal therapy on the micronuclei frequency, serum malondialdehyde and serum hs – CRP levels in chronic periodontitis patients with and without well controlled type 2 diabetes mellitus and healthy controls.

8. Scientific justification of the study:

- Oxidative stress induces cellular damage and insulin resistance, and emerges as the major mechanisms for related co-morbidities. Oxidative stress is intrinsically related to the pathogenesis of type 2 diabetes, dyslipidemia and PD, and it leads to DNA damage. One of the most established methods for evaluating DNA damage is the micronucleus (MN) test. The MN test analysis is based on the identification of a secondary nucleus.

- Polymorphonuclear leukocytes (PMNs) are the primary mediators of host response against pathogens in periodontal disease and they generate increased levels

of reactive oxygen species (ROS). The ROS-mediated tissue destruction could be measured by the final product of lipid peroxidation (LPO), such as malondialdehyde (MDA).

- High sensitive c – reactive protein is an acute phase protein which is elevated during inflammatory diseases and certain cardiovascular diseases.

9. Procedure for the study:

- The parents and subjects are explained about the research and their informed consent obtained for using blood sample (which were taken for routine examination for diabetes) for the study.
- Then you will be undergoing periodontal examination that involves the evaluation of gums as well as the amount of plaque present in the teeth.
- Collect fresh blood by venepuncture and transfer to heparinised vacutainers.
- Collected blood samples send on the same day to Genetika centre for advanced genetic studies, Pettah, Thiruvananthapuram, kerala. Collected blood samples will be discarded immediately after assessing the lab parameters.
- The confidentiality of the patient's data will also be maintained.

10. Expected risks for the participants:

Nil

11. Expected benefits of research for the participants:

- There is no direct benefit to you. The Collected 6ml blood sample will be used only for research purpose by a recognized Research Center **(**Genetika centre for advanced genetic studies, Pettah, Thiruvananthapuram, Kerala**)** without any loss of details regarding patient and sample collected.
- The study will help the health care practitioners to understand the role of non-surgical periodontal therapy on the micronuclei formation and elevated MDA levels in Type 2 diabetes mellitus and chronic periodontitis.
- Elevated concentrations of these markers were linked with a more frequent occurrence of cardiac events so early detection of these biomarkers help the clinician to plan the treatment thereby improving patient survival rate.

12. Maintenance of confidentiality:

a. You have the right to confidentiality regarding the privacy of your medical information

(Personal details, results of physical examinations, investigations, and your medical history)

b. By signing this document, you will be allowing the research team investigators, other study Personnel, sponsors, institutional ethics committee and any person or agency required by law to view your data, if required.

c. The results of study performed as part of this research may be included in your medical record.

d. The information from this study, if published in scientific journals or presented at scientific meetings, will not reveal your identity.

13. Why have I been chosen to be in this study?

a. Chosen because of grouping under the inclusion and exclusion criteria

b. Need of good sampling size

c. No invasive procedure that harm your health and it helps in diagnosis and helpful for the society

14. How many people will be in the study?

75

15. Agreement of compensation to the participants (In case of a study related injury):

No related injury anticipated. Patient will be taken care in case of complication and medical treatment will be provided.

16. Can I withdraw from the study at any time during the study period?

- The participation in this research is purely voluntary and you have the right to withdraw from this study at any time during the course of the study without giving any reasons.

- However, it is advisable that you talk to the research team prior to stopping information.

17. If there is any new findings/information, would I be informed?

Yes

18. Expected duration of the participant's participation in the study?

One year

19. Whom do I contact for further information?

For any study related queries, you are free to contact:

Dr. Blessing Emmanuel P,

Post graduate Student,

Department of Periodontics

Sree Mookambika Institute of Dental Sciences

Kulasekharam, KanyaKumari District-629161

Place:

Date: **Signature of Principal Investigator**

Signature of the participant

സമ്മത പത്രം - ഭാഗം - 1

പഠനവുമായി സഹകരിക്കുന്ന വ്യക്തികളുടെ അറിവിലേയ്ക്ക്

പ്രിയപ്പെട്ട സന്നദ്ധ സേവകൻ / സേവക,

ഞങ്ങൾ നിങ്ങളെ സ്വാഗതം ചെയ്യുന്നു. അതോടൊപ്പം ഈ പഠനവുമായി സഹകരിക്കാനുള്ള സന്നദ്ധതയോട് നന്ദി രേഖപ്പെടുത്തുന്നു. നിങ്ങൾ ഈ പഠനത്തിൽ പങ്കെടുക്കുന്നതിനു മുൻപ് ഈ പഠനം എന്തിനാണ് നടത്തപ്പെടുന്നത് എന്ന് അിറയേണ്ടതുണ്ട്. അതിനാൽ ഈ ഫോറത്തിൽ ഗവേഷണ പഠനത്തിന്റെ വിവരങ്ങളും മറ്റും വിശദമായി രേഖപ്പെടുത്തിയിരിക്കുന്നു. ഈ പഠനത്തിന്റെ രീതി, ഉദ്ദേശം, പ്രയോജനം, അപകടസാദ്ധ്യത, ക്ലേശം, മുൻകരുതൽ, എങ്ങനെ ഈ പഠനം മുൻപോട്ടു കൊണ്ടുപോകുന്നു എന്നിങ്ങനെ എല്ലാ വിവരങ്ങളും ഫോറത്തിൽ രേഖപ്പെടുത്തിയിരിക്കുന്നു. സദയം ഈ വിവരങ്ങൾ വായിച്ചു മനസ്സിലാക്കുവാൻ അഭ്യർത്ഥിക്കുന്നു. ഈ വിവരങ്ങളിൽ ശാസ്ത്രപരമായ പദങ്ങൾ ഉള്ളതിനാൽ സംശയനിവാരണത്തിനു പ്രധാന പഠനകർത്താവിനോടോ താഴെ രേഖപ്പെടുത്തി യിരിക്കുന്ന വ്യക്തികളോടോ ഫോറം ഒപ്പിടുന്നതിനു മുൻപോ അല്ലെങ്കിൽ ഈ പഠനത്തിന്റെ കാലാവധി തീരുന്നതുവരേയോ സമീപിക്കാവുന്നതാണ്.

1. മുഖ്യ ഗവേഷകൻ : ഡോ. ബ്ലസിങ് ഇമ്മാനുവ□ പി.

പോസ്റ്റുഗ്രാജുവേറ്റ്

ഡിപ്പാർട്ട്മെന്റ് ഓഫ് പെരിയോഡോന്റിക്സ്
ശ്രീ മൂകാംബിക ഇൻസ്റ്റിറ്റ്യൂട്ട് ഓഫ് ഡെന്റൽ സയൻസ്,

കുലശേഖരം - 629161

ഫോൺ: 7598197350

2. പ്രധാന മാർഗ്ഗദർശി : ഡോ.അരുൺ സദാശിവൻ

പ്രൊഫസർ

ഡിപ്പാർട്ട്മെന്റ് ഓഫ് പെരിയോഡോന്റിക്സ്
ശ്രീ മൂകാംബിക ഇൻസ്റ്റിറ്റ്യൂട്ട് ഓഫ് ഡെന്റൽ സയൻസസ്,
കുലശേഖരം.

ഫോൺ: 9847246961

3. സഹ മാർഗ്ഗ ദർശി : ഡോ. എലിസബെത്ത് കോശി

ഹെഡ് ഓഫ് ദി ഡിപ്പാർട്ട്മെന്റ്,

ഡിപ്പാർട്ട്മെന്റ് ഓഫ് പെരിയോഡോന്റിക്സ്
ശ്രീ മൂകാംബിക ഇൻസ്റ്റിറ്റ്യൂട്ട് ഓഫ് ഡെന്റൽ സയൻസസ്,
കുലശേഖരം.

ഫോൺ: 9447154335

4. ഇൻസ്റ്റിറ്റ്യൂട്ട് : **ശ്രീ. മൂകാംബിക ഇൻസ്റ്റിറ്റ്യൂട്ട് ഓഫ് ഡെന്റൽ സയൻസസ്**
പടനിലം, കുലശേഖരം,
കന്യാകുമാരി - 629161.
തമിഴ്നാട്.

5. പഠനത്തിന്റെ ശീർഷകം:

രക്തത്തി□ ഉള്ള മൈക്രോന്യൂക്ലിയ, മലോനാ□ഡിഹൈഡ്, എച്.സ്.സി.ആ□.പി എന്നിവയുടെ അളവുക□ പ്രമഹേം ഉള്ളവരും ഇല്ലാത്തവരുമായ പരിയോഡോന്റിറ്റിസ് രോഗികളി□: ഒരു ടസ്റ്റ് ട്യൂബ് പഠനം

6. പശ്ചാത്തല വിവരം ?

പരിയോഡോന്റിറ്റിസ് എന്ന രോഗം പല കാരണങ്ങളാ□ ഉണ്ടാകുന്നു എങ്കിലും പ്രധാന കാരണം കീടാണു ആണ്. പി. എം □സ് എന്ന കോശങ്ങ□ ഇ രോഗത്തിന് എതിരായി റിയാക്ടീവ് ഓക്സിജ□ സ്പീഷിസ് എന്ന രാസവസ്തു ഉണ്ടാക്കുന്നു . ഇവ ശരീരത്തി□ ഉണ്ടാക്കുന്ന ദോഷവശങ്ങ□ അറിയുന്നതിനായി ലിപിഡ് പറൊക്സിഡേഷ□ എന്ന പ്രക്രിയയുടെ അവസാന ഫലമായി ഉണ്ടാകുന്ന മാലോനാ□ഡിഹൈഡ് എന്ന രാസവസ്തുവിന്റെ അളവുക□ നമ്മെ സഹായിക്കുന്നു.ഓക്സിഡേറ്റീവ് സ്ട്രസ് ടൈപ്പ് 2 ഡയബറ്റിസ്, കൊലസ്റ്ററോ□ , പരിയോഡോന്റിറ്റിസ് എന്നീ രോഗങ്ങ□ക്ക് കാരണമായി ഡി. □. എ യെ നശിപ്പിക്കുന്നു.

7. ലക്ഷ്യങ്ങളും ഉദ്ദേശങ്ങളും

പ്രമഹേം ഉള്ളവരും ഇല്ലാത്തവരുമായ പരിയോഡോന്റിറ്റിസ് രോഗികളി□ താഴെ പറയുന്ന അളവുക□ കണ്ടുപിടിക്കുക.:

1. മൈക്രോന്യൂസ്ലയ്

2. മാലോനാ□ഡിഹൈഡ്

3. എച് സ് സി □ പി

8. ഗവേഷണം നടത്താനുള്ള ന്യായീകരണം

ഓക്സിഡേറ്റീവ് സ്ട്രസ് ടൈപ്പ് 2 ഡയബറ്റിസ്, കൊലസ്റ്ററോ□ , പരിയോഡോന്റിറ്റിസ് എന്നീ രോഗങ്ങ□ക്ക് കാരണമായി ഡി. □. എ യെ നശിപ്പിക്കുന്നു. ഈ ഡി □ എ നാശം അളക്കുന്നതിനായി മൈക്രോന്യൂസ്ലയ് ടസ്റ്റ് സഹായിക്കുന്നു.

പി. എം □സ് എന്ന കോശങ്ങ□ രോഗത്തിന് എതിരായി റിയാക്ടീവ് ഓക്സിജ□ സ്പീഷിസ് ഉണ്ടാക്കുന്നു. ഇവ ശരീരത്തി□ ഉണ്ടാക്കുന്ന ദോഷവശങ്ങ□ അറിയുന്നതിനായി ലിപിഡ് പറൊക്സിഡേഷ□ എന്ന പ്രക്രിയയുടെ അവസാന ഫലമായി ഉണ്ടാകുന്ന മാലോനാ□ഡിഹൈഡ് എന്ന രാസവസ്തുവിന്റെ അളവുക□ നമ്മെ സഹായിക്കുന്നു.

9. പഠന രീതി

നിങ്ങളുടെ മുഴുവ□ വിവരങ്ങളും അടങ്ങിയ കസേ ചാ□ട് രഖേപടുത്തുന്നതാവും. ആവശ്യമങ്കെി□ പരിശോധന നടത്തപ്പടുന്നതാണ്. പരിയോഡോന്റിയതിന്റെ പരിശോധന വളേയി□ **5** എം□ രക്തം ഒരു സിറിഞ്ചും നീഡിലും ഉപയോഗിച്ച് ശഖേരിക്കുന്നതാണ്. അതിനുശഷേം രക്തത്തിലെ പഞ്ചസാരയുടെ അളവും പ്ലാസ്മയിലെ മൈക്രോന്യൂസ്ലയ്, മാലോനാ□ഡിഹൈഡ്,എച് സ് സി □ പി എന്നീ വസ്തുക്കളുടെ അളവുക□ പരിശോധിക്കും.

പരിശോധനയ്ക്കു ശഷേം ശഖേരിച്ച രക്തവും താങ്കളെ കുറിച്ച് രഖേപ്പടെുത്തിയിരിക്കുന്ന ഒരു വിവരങ്ങളും ഒരു രീതിയിലും ചോ□ന്നുപോകാതെ ജനിറ്റിക്ക ലബോറട്ടറി, തിരുവനന്തപുരത്തു സൂക്ഷിക്കുന്നതായിരിക്കും.

10. പ്രതീക്ഷിക്കുന്ന അപകട സാദ്ധ്യതകൾ -

ഈ പഠനത്തിന്റെ പ്രവൃത്തനരീതി മൂലമുള്ള അപകട സാധ്യത വളരെ കുറവാണ്. മുറിവിൽ നിന്നോ, തൊലിക്കടിയിൽ നിന്നോ ഉള്ള ചെറിയ രക്തസ്രാവം, വീക്കം, ചെറിയ വേദന എന്നിവയാണ് അപകട സാധ്യതകൾ. ഇവ സുചിവച്ച സ്ഥലത്തിൽ ചെറിയ സമ്മർദ്ദം ചെലുത്തിയാൽ സാധൃകരിക്കപ്പെടും.

11. ഈ പഠനത്തിൽ പങ്കെടുക്കുന്നതുകൊണ്ട് എനിക്ക് എന്തെങ്കിലും ഉപയോഗമുണ്ടോ ?

താങ്ക□ക്കു നരേിട്ട് ഒരു ഉപയോഗവും ഇല്ല. ശഖേരിച്ച രക്തം പഠന ആവശ്യങ്ങ□ക്കായി ഉപയോഗിക്കപ്പടെുന്നതാരിക്കും.

12. ഞാൻ ഈ പഠനത്തിൽ പങ്കെടുക്കുന്ന വിവരം രഹസ്യമായി വയ്ക്കുമോ ?

നിങ്ങളിൽ നിന്ന് ശേഖരിക്കുന്ന എല്ലാ വിവരങ്ങളും രഹസ്യമായി വയ്ക്കുന്നതായിരിക്കും നിങ്ങളെ പറ്റിയുള്ള വിവരങ്ങൾ ആരോടും വെളിപ്പെടുത്തുന്നതായിരിക്കില്ല. ഭാവിയിൽ ഈ പഠനം ശാസ്ത്രലേഖനമായി പ്രസിദ്ധീകരിക്കുമ്പോൾ നിങ്ങളുടെ പേരിനു പകരം കോഡ് ആണ് ഉപയോഗിക്കുക.

13. എന്നെ എന്തുകൊണ്ട് ഈ പഠനത്തിൽ ഉൾപ്പെടുത്തി ?

നിങ്ങൾക്ക് പെരിഡോന്റൈസ് എന്ന അസുഖം ഉള്ളതുകൊണ്ട് നിങ്ങളെ ഈ പഠനത്തിൽ ഉൾപെടുത്തിയിരിക്കുന്നു.

14. എത്ര ആളുകൾ ഈ പഠനത്തിൽ ഉൾപ്പെടുന്നു. 75

15. നഷ്ടപരിഹാര ഉടമ്പടി

പഠനവിദേയമായി ഏതെങ്കിലും തരത്തിൽ രോഗം സങ്കീർണ്ണമായാൽ രോഗിയെ ഈ സ്ഥാപനത്തിൽ വിദഗ്ദചികിത്സയ്ക്ക് വിധേയനാക്കുന്നതാണ്.

16. ഏതെങ്കിലും വിധത്തിൽ വേതനം ലഭിക്കുമോ - ഇല്ല

17. എപ്പോൾ വേണമെങ്കിലും എനിക്ക് ഈ പഠനത്തിൽ നിന്ന് പിൻമാറാമോ

ഈ പഠനത്തിൽ പങ്കെടുക്കണമോ വേണ്ടയോ എന്നു തീരുമാനിക്കാനുള്ള പൂർണ്ണ അവകാശം നിങ്ങൾക്കുണ്ട്. നിങ്ങൾ ഇതിൽ പങ്കെടുക്കുവാൻ തീരുമാനിച്ചാൽ ഒരു സമ്മതപത്രത്തിൽ ഒപിട്ട് നൽകേണ്ടതുണ്ട്. ഈ പഠനത്തിൽ നിന്ന് ഏതു സമയവും പിൻവാങ്ങാനുള്ള സ്വാതന്ത്ര്യവും നിങ്ങൾക്കുണ്ട്. ഇത് നിങ്ങളുടെ മറ്റു ചികിത്സകളെ യാതൊരു വിധത്തിലും ബാധിക്കുന്നതായിരിക്കില്ല.

18. ഈ ഗവേഷണത്തിന്റെ ഫലമായി പുതിയ എന്തെങ്കിലും കണ്ടെത്തലുകളുണ്ടെങ്കിൽ അത് എന്നെ അിറയിക്കുമോ ?

ഈ പഠനത്തിന്റെ കണ്ടെത്തലുകൾ പഠന അവസാനം നിങ്ങളെ അിറയിക്കുന്നതായിരിക്കും.

19. ഈ പഠനത്തിന്റെ സമയ ദൈർഘ്യം എത്രയാണ് ?

ഏകദേശം മൂന്നു മാസം

20. കൂടുതൽ വിവരങ്ങൾക്കായി താഴെ പറയുന്നവരെ നിങ്ങൾക്ക് ബന്ധപ്പെടാവുന്നതാണ്.

ഡോ. ബ്ലസിങ് ഇമ്മാനുവ□ പി

പോസ്റ്റുഗ്രാജുവേറ്റ്

ഡിപ്പാർട്ട്മെന്റ് ഓഫ് പെരിയോഡോന്റിക്സ്

ശ്രീ മൂകാംബിക ഇൻസ്റ്റിറ്റ്യൂട്ട് ഓഫ് ഡെന്റൽ സയൻസ്,

കുലശേഖരം - 629161.

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സ്ഥലം:

തീയതി :

ஒப்புதல் வாக்குமூலம்

முதல் பாகம்

பங்கேற்பாளர்களுக்கு ஆய்வினை குறித்த தகவல்

அன்பார்ந்த பங்கேற்பாளர்களே,

இந்த ஆய்வில் பங்குபெற்றதற்கு உங்களை வரவேற்கிறோம்இதில் . இந்த ஆய்வு எதற்காக நடத்தப்படுகிறது என்பதை நீங்கள் பங்குபெறுவதற்கு முன்பு இந்த ஆய்வின் நன்மை தீமைகள் பற்றி இந்த படிவம் .அறிந்து கொள்ள வேண்டும் விளக்குகிறதுஇந்த ஆவணங்களை கவனமாக படித்து புரிந்து கொள்ளுதல் . இந்த ஆய்வில் பயன்படுத்தப்படும் அறிவியல் வார்த்தைகளில் உங்களுக்கு .அவசியம் ந்தேக இருக்குமென்றால் முதன்மை ஆய்வாளரை நேரிலோ அல்லது கீழே ச கொடுக்கப்பட்டுள்ள தொலைபேசி எண்ணிலோ அல்லது மின்னஞ்சல் வழியாகவோ தொடர்பு கொள்ளலாம்.

1. முதன்மை ஆய்வாளரின் பெயர்

டாக்டர். பிளசிங் இம்மானுவேல் பீ

முதுநிலை பல் மருத்துவ மாணவர்

பல் மற்றும் ஈறு நோய்பிரிவு

ஸ்ரீ மூகாம்பிகா பல் மருத்துவக் கல்லூரி

குலசேகரம்

2. வழிகாட்டியின் பெயர்

டாக்டர். அருண் சதாசிவன் எம்டிஎஸ்

பேராசிரியர்

பல் மற்றும் ஈறு நோய்பிரிவு

ஸ்ரீ மூகாம்பிகா பல் மருத்துவக் கல்லூரி

குலசேகரம்

3. இணை வழிகாட்டி

டாக்டர். எலிசபெத் கோஷி எம்டிஎஸ்

முதன்மை பேராசிரியர்

பல் மற்றும் ஈறு நோய்பிரிவு

ஸ்ரீ மூகாம்பிகா பல் மருத்துவக் கல்லூரி

குலசேகரம்

4. கல்லூரியின் பெயர்

ஸ்ரீ மூகாம்பிகா பல் மருத்துவக் கல்லூரி

குலசேகரம்

கன்னியாகுமரி மாவட்டம்

5. ஆராய்ச்சியின் தலைப்பு

"அறுவை சிகிச்சை இல்லா பெரியோடாண்டல் சிகிச்சையின் தாக்கத்தை மைக்ரோ நியூக்ளியை, ஆக்ஸிடேடிவ் ஸ்ட்ரெஸ் பாதிப்பை மேலனால்டிஹைடு மற்றும் சி ரியக்டிவ் ப்ரோடீன் மூலம் பெரியோடான்டிடிஸ் நோயாளிகள் மற்றும் பெரியோடான்டிடிஸ் மற்றும் நீரிழிவு வகை இரண்டு உள்ள நோயாளிகளிடமும் கண்டறிதல்"

6. குறிக்கோள்

டின்ஏ பாதிப்பை மைக்ரோ நியூக்ளியை மூலமும் ஆக்ஸிடேடிவ் ஸ்ட்ரெஸ் பாதிப்பை மேலனால்டிஹைடு மற்றும் சி ரியக்டிவ் ப்ரோடீன் மூலம் பெரியோடான்டிடிஸ் நோயாளிகள் மற்றும் பெரியோடான்டிடிஸ் மற்றும் நீரிழிவு வகை இரண்டு உள்ள நோயாளிகளின் ரத்தத்தில் கண்டறிதல்.

7. பின்புல தகவல்கள்

பெரியோடான்டிடிஸ் எனும் ஈறு நோயானது வாயில் தங்கும் நுண்ணுயிரிகளின் மூலம் உண்டாகிறதுஇதனை ஈடு செய்ய நமது உடலின் நோய் . எதிப்பு செல்கள் ரியக்டிவ் ஆக்சிஜன் ச்பெசீஸ் என்ற மூலக்கூற்றினை உண்டாக்குகின்றன இதன் மூலம் ஏற்படும் திசு பாதிப்பை .மேலனால்டிஹைடு என்ற மூலக்கூற்றினை கண்டறிவதின் மூலம் உறுதிப்படுத்தலாம் இந்த .ஆக்ஸிடேடிவ் ஸ்ட்ரெஸ் ,மற்றும் சி ரியக்டிவ் ப்ரோடீன் என்னும் புரதம் ஆகியவை நீரிழிவு , பெரியோடான்டிடிஸ் மற்றும் இருதய நோய் பாதிப்புகளில் முக்கிய பங்காற்றுகிறது.

8. ஆராய்ச்சியை அறிவியல் ரீதியாக உறுதிப்படுத்துதல்

பெரியோடான்டிடிஸ் மற்றும் நீரிழிவின் இரண்டாம் வகை ஆகிய இரு நோய்களும் டின்ஏ பாதிப்பு மற்றும் ஆக்ஸிடேடிவ் ஸ்ட்ரெஸ் பாதிப்பை ஏற்படுத்தக்கூடியது .

9. செய்முறை

✓ ஆய்வினைக் குறித்த ஓப்புதல் வாக்குமூலம் பெற்ற பிறகு நோயாளிகளிடம் இருந்து மற்றும் ஈறு பகுதிகளின் நிலை குறித்து மிலி இரத்தம் எடுக்கப்பட்டு பல் 5 .சோதனை செய்து தனிதனி படிவங்களில் பதிவு செய்யப்படும்

✓ இரத்தம் எடுத்த அன்றே அந்த ரத்தமானது திருவனந்தபுரத்தில் உள்ள ஜெனிடிகா மரபணு ஆய்வகத்திற்கு அனுப்பப்படும்.

10. எதிர்பார்க்கும் பக்கவிளைவுகள்

ஏதுமில்லை

11. ஆராய்ச்சியில் பங்கு பெறுபவர்களுக்கு கிடைக்கும் நன்மைகள்

உங்களுக்கு நேரடி நன்மைகள் கிடைக்கப்பெறவில்லை என்றாலும் உங்களிடம் இருந்து எடுக்கப்படும் ரத்தம் மரபணு குறித்த ஆய்விற்கும் எதிர்கால கண்டுபிடிப்பிற்கும் பயன்படும். இதில் கண்டறியப்படும் காரணிகள் அதிகரிக்கும்போது இருதய நோய்கள் ஏற்பட வாய்ப்புகள் அத்கம் என்பதால் இந்த காரணிகளை முன்கூட்டியே கண்டறிவதின் மூலம் இருதய நோய்களை தடுக்கலாம்.

12. தங்களைப் பற்றிய விவரங்கள் அனைத்தும் பாதுகாக்கப்படுமா?

உங்களின் தகவல்கள் அனைத்தும் பாதுகாக்கப்படும் இந்த படிவத்தில் நீங்கள் ஒப்பு .

உங்கள் தகவல்களை இடுவதின் மூலம்முதன்மை ஆய்வாளரோ அல்லது வழிகாட்டியோ தேவைப்பட்டால் எடுத்து பார்க்க முடியும்இந்த ஆய்வின் முடிவுகள் . வருங்காலத்தில் அறிவியல் கருத்தரங்குகளிலோ அல்லது அறிவியல் நாளேடுகளிலோ உங்கள் தனிப்பட்ட தகவல்கள் தவிர்த்து பதிவு செய்யப்படலாம் .

13. எதற்காக நான் இந்த ஆராய்ச்சிக்கு தேர்ந்தெடுக்கப்பட்டேன்?

உங்களுக்கு பெரியோடான்டிடிஸ் மற்றும் நீரிழிவின் இரண்டாம் வகை ஆகிய இரு நோய்களும் அல்லது இவைகளில் ஏதேனும் ஒன்று இருப்பதால் தேர்ந்தெடுக்கப்பட்டுள்ளீர்கள்.

14. **இந்த ஆராய்ச்சியில் எத்தனைநபர்கள் பங்குபெறுகிறார்கள்?**

75 நபர்கள் பங்குபெறுகிறார்கள்

15. இந்த ஆராய்ச்சியில் ஏதேனும் தீங்கு ஏற்ப்பட்டால் அது எவ்வாறு ஈடு செய்யப்படும் ?

இந்த ஆராய்ச்சியில் ஏதேனும் தீங்கு ஏற்ப்பட்டால் அதற்கான மருத்துவ செலவை முதன்மை ஆய்வாளர் ஏற்றுக்கொள்வார் .

16. இந்த ஆராய்ச்சியில் ஏதேனும் தீங்கு ஏற்ப்பட்டால் இடையில் விலகிக்கொள்ளலாமா?

தங்களுக்கு இந்த ஆராய்ச்சியில் பங்கு பெறவும் விலகிக்கொள்ளவும் முழு சுதந்திரம் உண்டு இதில் இருந்து விலகிக்கொள்வதால் உங்களுக்கு எந்த தீங்கும் . .ஏற்படாது

17. இந்த ஆராய்ச்சியில் ஏதேனும் புதிதாக கண்டுபிடிக்கப்பட்டால் எனக்கு அறிவிக்கப்படுமா?

ஆம் இந்த ஆராய்ச்சியில் ஏதேனும் புதிதாக கண்டுபிடிக்கப்பட்டால் . .உங்களுக்கு கண்டிப்பாக அறிவிக்கப்படும்

18. எத்தனை நாட்கள் இந்த ஆராய்ச்சியில் பங்கு பெற வர வேண்டும்?

இரண்டு முறை மட்டும் வந்தால் போதும்.

19. மேற்கொண்டு நான் தொடர்பு கொள்ள என்ன செய்ய வேண்டும் ?

ஆய்வை பற்றிய சந்தேகங்களுக்கு தொடர்பு கொள்ள வேண்டிய முகவரி:

டாக்டர். பிளசிங் இம்மானுவேல் பீ

முதுநிலை பல் மருத்துவ மாணவர்

பல் மற்றும் ஈறு நோய்பிரிவு

ஸ்ரீ மூகாம்பிகா பல் மருத்துவக் கல்லூரி

குலசேகரம் - 629161

இடம் முதன்மை ஆராய்ச்சியாளரின் கையொப்பம்

நாள்

CONSENT FORM

PART 2 OF 2

PARTICIPANTS CONSENT FORM

The details of the study have been explained to me in writing and the details have been fully explained to me. I confirm that I have understood the study and had the opportunity to ask questions. I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without the medical care that will normally be provided by the hospital being affected. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s). I have been given an information sheet giving details of the study. I fully consent to participate in the study titled: "EFFECT OF NON SURGICAL PERIODONTAL THERAPY ON THE MICRONUCLEI FREQUENCY, SERUM MALONDIALDEHYDE AND SERUM HIGH SENSITIVE C – REACTIVE PROTEIN LEVELS IN CHRONIC PERIODONTITIS PATIENTS WITH AND WITHOUT WELL CONTROLLED TYPE 2 DIABETES MELLITUS"

Serial no / Reference no:

Name of the participant:

Address of the participant:

Signature / thumb impression of the Parent / Legal guardian

Witnesses:

1.

2.

Date:

Place:

സമ്മതപത്രം

ഭാഗം - 2

ഈ പഠനത്തെ പറ്റിയുള്ള എല്ലാ കാര്യങ്ങളും എനിക്ക് പറഞ്ഞ് മനസ്സിലാക്കി തരികയും അതിന്റെ ഒരു പകർപ്പ് എനിക്കു നൽകുകയും ചെയ്തിട്ടുണ്ട്. ഈ പഠനം ഗവേഷണത്തിനായി ഉള്ളതാണെന്നും എനിക്ക് ഇതിൽ നിന്ന് നേരിട്ട് ഒരു ഫലവും ഉണ്ടാകില്ലെന്നും ഞാൻ മനസ്സിലാക്കുന്നു. ഈ പഠനത്തിന്റെ രീതിയും ഉദ്ദേശവും എനിക്ക് മനസ്സിലാക്കി തന്നിട്ടുണ്ട്. അതു പോലെ എനിക്ക് സംശയങ്ങൾ ചോദിക്കാൻ അവസരങ്ങൾ ലഭിച്ചിട്ടുമുണ്ട്. ഇതിൽ പങ്കെടുക്കാനും പങ്കെടുക്കാതിരിക്കാനും ഉള്ള അവകാശം എനിക്കുണ്ടെന്നും അതുപോലെ പഠനത്തിന്റെ ഏതു ഘട്ടത്തിലും ഇതിൽ നിന്ന് പിൻവങ്ങാനുള്ള സ്വാതന്ത്ര്യവും എനിക്കുണ്ടെന്ന് ഞാൻ മനസ്സിലാക്കുന്നു. ഈ പഠനത്തിൽ പങ്കെടുക്കുന്നതുകൊണ്ടോ, പങ്കെടുക്കാത്തതുകൊണ്ടോ എന്റെ മറ്റു ചികിത്സകളെ ബാധിക്കുന്നതല്ലെന്ന് ഞാൻ അിറയുന്നു. "രക്തത്തി□ ഉള്ള മൈക്രോന്യൂക്ളിയ, മലോനാ□ഡിഹൈഡ്, എച്ച് സി ആ□ പി എന്നിവയുടെ അളവുക□ പ്രമേഹം ഉള്ളവരും ഇല്ലാത്തവരുമായ പരിയോഡോന്റിറിസ് രോഗികളി□: ഒരു ടസ്റ്റ് ട്യൂബ് പഠനം**" **എന്ന ഗവേഷണത്തിൽ പങ്കെടുക്കുന്നതിനും ഇതിന്റെ ഫലങ്ങൾ ശാസ്ത്രലേഖനത്തിൽ പ്രസിദ്ധീകരിക്കുന്നതിനും എനിക്ക് സമ്മതമാണെന്ന് ഞാൻ ഇതിനാൽ അിറയിച്ചുകൊളളുന്നു.**

സീരിയൽ നമ്പർ / റഫറൻസ് നമ്പർ :

പങ്കെടുക്കുന്ന ആളിന്റെ പേര് :

മേൽവിലാസം :

ഫോൺ നമ്പർ :

ഒപ്പ് / വിരലടയാളം

സാക്ഷി :

സ്ഥലം :

തീയതി

ஒப்புதல் படிவம்

இரண்டாம் பாகம்

பங்கேற்பாளரின் ஒப்புதல்

இந்த ஆய்வின் தகவல்கள் அனைத்தும் எனக்கு வாய் மொழியாகவும் எழுத்து மொழியாகவும் தெரிவிக்கப்பட்டதுஇந்த ஆய்வில் நானாக முன்வந்து . இதன் மூலம் எனக்கு நேரடி நன்மைகள் .பங்குபெறுகிறேன் என அறிவிக்கிறேன் இல்லை எனினும் இந்த ஆய்வின்முடிவு மருத்துவத்துறையின் முன்னேற்றத்திற்கு பயன்படும் என்பதை அறிந்து இதற்கு சம்மதிக்கிறேன்எனக்கு இந்த ஆய்வைக் . .விரிவான தகவல்கள் அடங்கிய படிவம் தரப்பட்டுள்ளது குறித்த

நான் "**அறுவை சிகிச்சை இல்லா பெரியோடாண்டல் சிகிச்சையின் தாக்கத்தை மைக்ரோ நியூக்ளியை, ஆக்ஸிடேடிவ் ஸ்ட்ரெஸ் பாதிப்பை மேலனால்டிஹைடு மற்றும் சி ரியக்டிவ் ப்ரோடீன் மூலம் பெரியோடான்டிடிஸ் நோயாளிகள் மற்றும் பெரியோடான்டிடிஸ் மற்றும் நீரிழிவு வகை இரண்டு உள்ள நோயாளிகளிடமும் கண்டறிதல் "**என்ற இந்த ஆய்வில் பங்கேற்க முழு மனதுடன் சம்மதிக்கிறேன் .

குறிப்பு எண்

பெயர் கையொப்பம்கைரேகை /
பதிவு

முகவரி

தொலைபேசி எண்

சாட்சி .1

.2

CASE RECORD FORM

"EFFECT OF NON SURGICAL PERIODONTAL THERAPY ON THE MICRONUCLEI FREQUENCY, SERUM MALONDIALDEHYDE AND SERUM HIGH SENSITIVE C – REACTIVE PROTEIN LEVELS IN CHRONIC PERIODONTITIS PATIENTS WITH AND WITHOUT WELL CONTROLLED TYPE II DIABETES MELLITUS"				
Name:				
Address:				
Age		Sex	Male	Female
Residence	Rural	Urban	Coastal	
Educational Level:	Primary	Secondary	Higher Secondary	Graduate/Post graduate
Occupational Type:	Sedentary		Non-sedentary	
Socio-economic status:	Low	Average	High	
Height:				
Weight:				
Abdominal Circumference				
BMI				
Obesity	Yes		No	
Regular exercise	Yes		No	
Physical activity	Good	Average	Poor	
Smoking	Yes		No	
Alcohol Consumption	Yes		No	
Habit of Chewing	Yes		No	
Use of Medication/drugs/pills	Yes		No	
Frequency of brushing (per day)	1	2	>2	
Use of tooth paste	Yes		No	
Use of mouthwash	Yes		No	
Bleeding gum during brushing	Yes		No	
Sensitive teeth	Yes		No	
Family H/o gum problem	Yes		No	
Dental prosthesis	Fixed	Removable	None	
Mental stress	Yes		No	
H/o Diabetes	Yes		No	
H/o Hypertension	Yes		No	
H/o Dyslipidemia	Yes		No	
H/o CAD	Yes		No	
Diet	Vegetarian		Non-Vegetarian	

INVESTIGATIONS

FBS	
RBS	
HbA1c	
Total Cholesterol	
MDA	
CBMN frequency	
Hs - CRP	

PERIODONTAL PARAMETERS

PLAQUE INDEX

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28

48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

Plaque index score:

GINGIVAL INDEX:

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28

48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

Gingival Index Score:

PROBING POCKET DEPTH

48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28

GINGIVAL RECESSION/ GINGIVAL ENLARGEMENT

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28

48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

CLINICAL ATTACHMENT LEVEL

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28

48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

Signature of Investigator

Group wise clinical parameters obtained. (Pre-Operative data)

Patient code for Group 1 - A1 - A28

Patient code for Group 2 - B1 - B27

Patient code for Group 3 - C1 - C28

PATIENT CODE	AGE	PI SCORE	GI SCORE	MEAN PPD	MEAN CAL
A1	45	1.9	2.1	4.87	4.9
A2	37	2.1	2.3	4.8	5.02
A3	39	1.8	1.9	4.28	4.56
A4	51	1.6	1.8	4.36	4.67
A5	58	1.38	1.9	4.5	4.76
A6	41	1.7	2.1	4.3	4.64
A7	48	1.4	2.3	4.38	4.68
A8	58	1.6	1.8	4.89	5.2
A9	54	1.8	2.1	4.65	5.21
A10	49	1.6	1.7	4.72	5.1
A11	41	2.1	2.3	4.28	4.5
A12	40	1.8	2.1	4.87	5.05
A13	46	1.7	1.6	4.62	4.95
A14	51	1.5	1.7	4.3	4.5
A15s	38	1.1	1.2	4.32	4.65
A16	50	1.4	1.5	4.11	4.2
A17	38	1.5	1.7	4.28	4.5
A18	56	1.9	2.1	4.64	5.04
A19	48	1.8	1.8	4.9	5.3
A20	46	1.7	2.03	4.3	4.67
A21	53	1.7	1.8	4.25	4.68
A22	58	1.8	1.6	4.86	5.2
A23	54	1.7	1.8	4.82	5.3
A24	51	1.74	1.87	4.07	4.4
A25	58	1.31	1.48	3.64	3.96

A26	49	1.8	1.87	3.97	4.43
A27	41	1.42	1.67	5.48	5.7
A28	58	1.75	1.87	4.5	4.86
B1	31	1.9	2.4	3.92	4.08
B2	45	2.2	2.4	3.62	4.17
B3	43	1.6	1.8	4.06	4.85
B4	33	1.9	2.1	3.72	4.06
B5	44	1.3	1.9	3.73	4.82
B6	38	2.1	2.3	4.11	4.76
B7	39	1.2	1.4	3.4	3.4
B8	38	1.6	1.8	3.64	3.82
B9	31	1.6	1.7	3.9	4.12
B10	39	2.1	2.3	4.1	4.3
B11	30	1.9	2.1	3.8	3.8
B12	33	1.6	1.9	3.43	3.7
B13	34	1.65	1.8	3.74	3.93
B14	34	1.43	1.5	3.38	3.63
B15	31	1.3	1.4	4.2	4.5
B16	31	1.4	1.4	4.36	4.86
B17	43	1.5	1.64	4.28	4.51
B18	44	1.42	1.5	3.84	4.45
B19	38	1.5	1.5	3.84	4.64
B20	48	1.4	1.6	4.2	4.67
B21	31	1.6	1.5	3.9	4.4
B22	30	1.5	1.5	3.79	4.1
B23	33	1.5	1.6	3.68	4.2
B24	34	1.5	1.5	3.75	4.14
B25	38	1.5	1.3	4.1	4.52
B26	39	1.5	1.51	3.76	4.8
B27	45	1.8	1.75	4.2	4.8

C1	31	1.2	0.9	2.3	2.3
C2	35	1.1	1.1	2.1	2.1
C3	32	1.2	0.8	2.23	2.23
C4	36	1.6	1.8	2.56	2.56
C5	36	1.4	1.4	2.3	2.3
C6	35	1.3	1.3	2.5	2.5
C7	30	1.1	0.8	2.38	2.38
C8	30	1.1	0.9	2.4	2.4
C9	34	1.45	1.77	2.43	2.43
C10	35	1.3	1.1	2.42	2.42
C11	34	1.1	0.9	2.1	2.1
C12	31	1.5	1.6	2.8	2.8
C13	36	1.72	1.65	2.52	2.52
C14	32	1.5	1.43	2.5	2.5
C15	38	1.39	1.35	2.79	2.79
C16	31	1.25	1.3	2.85	2.85
C17	31	1.27	1.43	2.65	2.65
C18	31	1.73	1.44	2.69	2.69
C19	30	1.36	1.54	2.68	2.68
C20	36	1.17	1.3	2.6	2.6
C21	31	1.125	1.32	2.68	2.68
C22	33	1.4	1.8	2.39	2.39
C23	30	1.14	1.15	2.57	2.57
C24	31	1.07	1.14	2.63	2.63
C25	32	1.46	1.45	2.32	2.32
C26	31	1.37	1.44	2.55	2.55
C27	36	1.39	1.45	2.61	2.61
C28	30	1.37	1.39	2.82	2.82

Group wise laboratory parameters obtained. (Pre-Operative data)

Patient code for Group 1 - A1 - A28

Patient code for Group 2 - B1 - B27

Patient code for Group 3 - C1 - C28

PATIENT CODE	RBS	Total Cholestrol	Triglyceride	HbA 1C	hsCRP	MDA	Mean CBMN frequency
A1	113	230	304	6.12	8.65	3.2	12.26
A2	122	245	312	6.8	9.5	3.85	13.9
A3	156	259	320	6.95	9.2	3.41	13.4
A4	110	219	275	6.12	8.56	3.35	12.6
A5	175	210	338	6.85	9.45	3.95	13.86
A6	136	223	295	6.47	9.36	3.69	13.56
A7	148	258	224	6.56	8.9	3.4	13.03
A8	158	249	315	5.95	7.59	3.25	13.25
A9	196	263	313	6.1	8.41	3.9	13.8
A10	147	258	298	6.11	8.25	3.41	13.48
A11	126	206	186	6.58	8.95	3.2	12.45
A12	131	233	195	6.87	8.74	3.25	12.49
A13	163	287	302	6.21	8.66	3.69	13.59
A14	150	280	315	5.93	7.39	3.25	12.48
A15-	115	210	204	6.57	8.91	3.14	12.21
A16	157	218	285	6.52	8.75	3.59	13.6
A17	127	240	186	6.3	8.12	3.28	12.58
A18	144	266	175	6.29	9.12	3.47	13.4
A19	136	254	185	6.41	8.96	3.74	13.7
A20	174	270	310	6.89	9.19	3.56	13.45
A21	186	237	195	6.12	8.12	3.47	13.5
A22	125	225	296	6.34	8.88	3.69	13.57
A23	162	238	269	6.45	8.74	3.5	13.5

A24	185	230	285	6.88	9.58	3.4	13.8
A25	146	256	214	6.71	8.66	3.1	12.9
A26	131	220	176	6.95	9.21	2.9	13.2
A27	147	236	158	6.66	8.39	2.65	12.58
A28	139	213	247	6.99	9.35	3.4	13.7
B1	88	125	85	4.36	6.54	2.59	11.56
B2	110	136	92	4.88	6.95	2.54	11.97
B3	86	185	99	5.96	7.24	2.6	12.6
B4	85	156	108	5.34	7.36	2.6	12.6
B5	112	191	112	4.55	7.89	3.2	12.16
B6	96	175	96	5.98	7.64	3.85	12.89
B7	98	201	88	4.69	7.12	2.96	12.01
B8	95	186	114	5.15	7.58	3.69	12.63
B9	103	159	108	4.35	7.06	2.96	12.35
B10	89	150	112	4.96	6.32	2.15	11.9
B11	96	129	102	4.78	7.45	2.84	12.25
B12	85	125	102	4.52	6.87	2.6	11.6
B13	91	145	124	5.36	7.14	2.63	12.64
B14	89	204	94	4.4	7.19	2.14	12.3
B15	96	161	83	4.63	6.23	3.12	11.5
B16	110	171	112	4.12	6.12	2.74	11.96
B17	95	185	115	5.71	7.2	3.12	12.5
B18	100	201	136	5.69	7.14	2.55	12.41
B19	79	210	148	4.88	6.39	2.1	11.6
B20	98	182	105	4.17	7.09	2.78	12.01
B21	101	165	136	5.48	7.31	2.96	12.8
B22	86	149	112	4.26	6.4	2.5	11.6
B23	96	180	115	4.85	6.21	2.48	11.7
B24	89	174	126	4.56	7.28	1.6	12.01
B25	91	156	157	4.32	6.19	2.91	11.9

B26	86	145	114	5.52	7.11	2.4	12.4
B27	96	175	186	4.3	6.11	3.2	11.3
C1	63	145	135	3.56	1.25	0.85	10.36
C2	107	172	115	4.52	1.96	0.86	10.2
C3	107	186	115	3.63	0.96	1.02	9.33
C4	105	114	114	4.11	1.47	0.89	10.08
C5	95	123	120	3.28	1.36	0.89	10.2
C6	96	142	89	3.75	1.45	0.86	10.7
C7	75	115	124	3.69	1.55	1.03	10.25
C8	83	121	105	3.33	1.74	1.14	10.4
C9	98	128	113	4.15	1.85	0.9	10.64
C10	95	158	128	3.96	1.36	0.85	10.36
C11	75	115	93	3.14	0.945	0.96	9.4
C12	77	163	125	3.3	1.12	0.86	9.8
C13	95	161	96	3.19	0.95	1.02	9.33
C14	85	120	105	3.74	0.85	1.12	9.21
C15	75	179	99	3.51	0.896	1.3	9.6
C16	78	195	144	3.47	0.86	1.69	9.85
C17	86	168	118	3.88	0.95	1.5	9.5
C18	105	176	122	3.56	1.3	1.45	9.63
C19	95	150	94	3.89	1.66	1.45	10
C20	75	134	109	3.15	0.94	0.95	9.5
C21	81	153	124	3.78	0.88	1.23	9.5
C22	100	186	124	3.45	0.96	1.4	9.14
C23	94	168	110	3.87	1.49	0.96	10
C24	94	175	106	3.1	0.89	0.89	9.7
C25	95	142	115	3.26	0.99	1.63	9.2
C26	109	136	95	3.17	1.11	1.69	9.56
C27	83	195	124	3.22	0.85	1.78	9.3
C28	105	174	102	3.19	1.54	0.89	10.9

Group wise clinical parameters obtained. (Post-Operative data)

Patient code for Group 1 - A1P - A28P (Lost follow-up A4-A6)

Patient code for Group 2 – B3P - B27P (Lost follow-up B1-B2)

Patient code for Group 3 – C4P - C28 (Lost follow-up C1-C3)

PATIENT CODE	PI SCORE	GI SCORE	MEAN PPD	MEAN CAL
A1P	0.5	0.64	2.63	3.01
A2P	0.42	0.32	2.5	2.7
A3P	0.23	0.25	2.5	2.78
A7P	0.67	0.9	3.5	3.61
A8P	0.4	0.6	2.7	3.1
A9P	0.42	0.65	2.32	2.65
A10P	0.32	0.4	2.6	3.1
A11P	0.5	0.52	2.32	2.45
A12P	0.32	0.36	2.8	3.2
A13P	0.4	0.41	2.6	2.9
A14P	0.2	0.4	2.2	2.9
A15P	0.23	0.32	2.4	2.65
A16P	0.31	0.2	2.5	2.63
A17P	0.1	0.5	2.4	2.98
A18P	0.23	0.24	2.9	3.1
A19P	0.4	0.32	2.4	2.85
A20P	0.64	0.9	3.2	3.75
A21P	0.32	0.25	2.45	3.1
A22P	0.23	0.31	2.7	3.1
A23P	0.23	0.37	2.73	3.1
A24P	0.43	0.32	2.7	3.2
A25P	0.23	0.26	2.2	2.47
A26P	0.23	0.22	2.41	2.78
A27P	0.27	0.32	2.6	3.1

A28P	0.31	0.32	2.7	3.2
B3P	0.36	0.42	2.41	2.63
B4P	0.32	0.34	2.72	2.9
B5P	0.26	0.36	2.25	2.63
B6P	0.5	0.9	2.46	3.1
B7P	0.23	0.25	2.45	2.74
B8P	0.26	0.52	2.65	2.63
B9P	0.23	0.26	2.72	2.9
B10P	0.3	0.4	2.7	2.9
B11P	0.25	0.23	2.12	2.54
B12P	0.3	0.72	2.46	3.1
B13P	0.23	0.52	2.72	3.1
B14P	0.32	0.29	2.62	2.9
B15P	0.2	0.5	2.3	2.45
B16P	0.23	0.32	2.5	2.7
B17P	0.45	0.32	2.63	2.9
B18P	0.21	0.24	2.12	2.54
B19P	0.46	0.8	2.21	2.54
B20P	0.32	0.37	2.63	2.78
B21P	0.25	0.27	2.38	2.38
B22P	0.45	0.32	2.21	2.54
B23P	0.32	0.34	2.45	2.74
B24P	0.9	0.8	2.67	2.9
B25P	0.25	0.23	2.3	2.8
B26P	0.24	0.26	2.7	2.9
B27P	0.21	0.27	2.6	2.9
C4P	0.28	0.34	2.6	2.6
C5P	0.32	0.23	2.31	2.31
C6P	0.22	0.24	2.51	2.51
C7P	0.25	0.28	2.72	2.72

C8P	0.31	0.37	2.7	2.7
C9P	0.23	0.26	2.4	2.4
C10P	0.4	0.32	2.8	2.8
C11P	0.23	0.25	2.5	2.5
C12P	0.2	0.28	2.8	2.8
C13P	0.23	2.24	2.72	2.72
C14P	0.31	0.28	2.5	2.5
C15P	0.23	0.24	2.3	2.3
C16P	0.24	0.26	2.5	2.5
C17P	0.32	0.31	2.6	2.6
C18P	0.27	0.31	2.8	2.8
C19P	0.23	0.28	2.8	2.8
C20P	0.31	0.29	2.45	2.45
C21P	0.3	0.29	2.3	2.3
C22P	0.23	0.25	2.4	2.4
C23P	0.25	0.25	2.3	2.3
C24P	0.5	0.5	2.1	2.1
C25P	0.24	0.31	2.5	2.5
C26P	0.23	0.27	2.6	2.6
C27P	0.22	0.23	2.45	2.45
C28P	0.23	0.24	2.2	2.2

Group wise laboratory parameters obtained. (Post-Operative data)

Patient code for Group 1 - A1P - A28P (Lost follow-up A4-A6)

Patient code for Group 2 – B3P - B27P (Lost follow-up B1-B2)

Patient code for Group 3 – C4P - C28 (Lost follow-up C1-C3)

PATIENT CODE	RBS	Total Cholestrol	Triglyceride	HbA1C	hsCRP	MDA	Mean CBMN frequency
A1P	105	205	274	5.96	5.24	2.5	12.15
A2P	113	208	256	6.35	6.39	1.46	13.12
A3P	148	194	277	6.55	6.14	2.6	13.01
A7P	120	210	210	6.12	5.19	2.63	13.01
A8P	149	229	245	5.56	4.12	1.58	13.1
A9P	150	241	236	5.74	5.46	2.95	13.12
A10P	138	245	240	5.85	5.25	2.15	13.27
A11P	121	189	152	6.17	5.47	2.74	12.33
A12P	135	238	198	6.32	8.96	3.35	12.52
A13P	117	201	279	5.89	5.12	2.36	13.13
A14P	85	198	236	5.24	4.36	1.47	12.2
A15P	112	208	196	6.32	5.96	2.45	12.05
A16P	146	212	240	6.12	5.47	2.95	12.49
A17P	130	245	189	5.89	8.56	3.46	12.6
A18P	140	245	172	5.74	6.25	2.49	12.06
A19P	132	236	181	6.12	5.21	2.88	13.2
A20P	166	156	290	6.21	6.39	2.33	13.38
A21P	110	210	152	5.89	5.36	2.47	12.96
A22P	120	210	288	5.94	5.14	2.54	13.13
A23P	168	240	272	6.13	8.9	3.74	13.64
A24P	170	215	280	6.29	3.47	2.14	13.2
A25P	120	180	195	6.33	5.49	2.71	12.46
A26P	127	215	175	6.48	6.12	1.98	12.8
A27P	132	126	154	6.22	5.12	1.77	12.12

A28P	130	209	240	6.49	6.47	2.94	13.06
B3P	81	180	96	5.12	4.8	2.15	12.4
B4P	114	188	99	4.96	4.1	2.4	12.44
B5P	101	179	109	4.12	4.36	2.6	12.02
B6P	97	181	94	5.56	4.25	2.98	12.63
B7P	101	206	91	4.12	7.41	3.1	12.25
B8P	90	174	110	4.86	4.55	2.88	12.46
B9P	93	153	102	4.12	4.36	2.56	12.17
B10P	85	149	110	4.44	3.26	1.85	11.61
B11P	91	127	98	4.21	4.12	2.41	12.11
B12P	84	124	93	4.36	3.33	2.12	11.24
B13P	90	155	120	5.33	4.78	2.47	12.39
B14P	70	172	90	4.21	4.18	1.98	12.04
B15P	88	146	80	4.6	3.45	2.95	11.32
B16P	97	169	110	4.1	3.17	2.11	11.44
B17P	85	180	112	5.56	4.52	2.47	12.31
B18P	98	188	125	5.54	4.16	2.48	12.14
B19P	76	195	139	4.65	3.16	1.54	11.12
B20P	105	186	110	4.1	7.25	2.96	12.24
B21P	92	156	132	5.33	4.32	2.36	12.14
B22P	100	168	110	4.44	3.22	2.2	11.25
B23P	91	177	109	4.8	3.14	2.54	11.15
B24P	84	165	120	4.56	4.55	0.92	11.45
B25P	88	154	149	4.12	3.18	2.14	11.39
B26P	84	140	110	5.12	4.12	1.95	12.01
B27P	93	173	176	4.12	3.78	2.8	11.06
C4P	98	110	110	4.1	1.33	0.56	10.01
C5P	94	121	120	3.27	1.29	0.85	9.56
C6P	91	140	86	3.14	1.42	0.51	10.69
C7P	78	118	129	3.66	1.59	1.12	10.45

C8P	80	118	97	3.13	1.63	1.12	10.32
C9P	95	121	110	4.15	1.79	0.83	10.12
C10P	72	144	124	3.97	1.28	0.81	10.24
C11P	75	110	94	3.13	0.93	0.87	9.12
C12P	72	156	119	3.4	1.1	0.86	9.32
C13P	94	150	95	3.18	0.91	0.99	9.31
C14P	89	122	112	3.77	0.86	1.25	9.54
C15P	84	169	85	3.5	0.79	1.01	9.47
C16P	76	188	130	3.44	0.84	1.48	9.41
C17P	84	162	114	3.86	0.91	1.22	9.46
C18P	99	170	120	3.51	1.28	1.03	9.51
C19P	91	145	91	3.88	1.63	1.14	9.48
C20P	73	120	104	3.15	0.87	0.91	9.43
C21P	83	158	129	3.79	0.91	1.32	9.67
C22P	94	174	121	3.46	0.92	1.03	9.09
C23P	92	158	107	3.85	1.44	0.88	9.87
C24P	90	170	93	3.11	0.84	0.71	9.5
C25P	93	133	112	3.29	0.93	1.16	9.19
C26P	105	118	94	3.5	0.85	1.42	9.42
C27P	82	184	113	3.21	0.81	1.55	9.29
C28P	98	170	97	3.17	1.14	0.73	10.45